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PERSISTENCE OF INFLUENZA VIRUS ON THE HUMAN HAND*

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THE persistence of potent influenza virus in dried extraembryonic fluids of infected chick embryos reported in a preceding paper[1] of this series in conjunction with the earlier convincing report of Edward[2] regarding the persistence of influenza virus on soiled utensils at once suggested the possible agency of the human hand in the dissemination of this virus, particularly the hand which comes into contact with food and with utensils for serving food. The matter has already been studied by Krueger and his associates,[3] who observed rapid loss of potency in virus deposited on the hand and therefore concluded that transmission through this agency is not apt to be an important hazard.

Because contamination with the bacteria of the skin was to be expected in these experiments it was decided to use white mice rather than embryonated eggs in testing for active virus. The mice ranged in weight from 10 to 20 grams. Some were Swiss mice and others were unspecified white mice from dealers in laboratory animals. All seemed satisfactory for the tests. Inoculation was done by instilling several drops of virus suspension into the nostrils of the mouse under light ether anesthesia. A blunt 24 gauge needle attached to a tuberculin syringe was used.

Experiment 1, January 20, 1943.—Influenza virus of the Melbourne A strain which had been propagated in embryonated eggs was passed twice in series through mice. The lungs of a mouse of the second passage were ground and suspended in saline solution, allowed to settle, and the opalescent super-

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natant fluid was removed and inoculated directly, without drying, intranasally to four mice. All survived to be sacrificed on the ninth day. One mouse showed consolidation of three pulmonary lobes ($9K_3$), one had two consolidated lobes ($9K_2$), and two had one consolidated lobe ($9K_1$). Some of the virus suspension was dried on glass by spreading 0.2 c.c. of it over the bottom of each of two sterile Petri dishes. After remaining dry for twenty minutes the material in one dish was taken up in 0.4 c.c. saline solution and inoculated intranasally to four mice. These were killed on the ninth day with positive findings; one mouse had small solid spots in the lungs ($9K_+$), one had one lobe consolidated ($9K_1$), another had two ($9K_2$), and another had three consolidated lobes ($9K_3$). The material dried in the other dish was kept for forty-eight hours and then resuspended in 0.4 c.c. saline solution and given to four mice intranasally. These were killed on the ninth day and revealed no evidence of infection ($9K_0$, $9K_0$, $9K_0$, $9K_0$).

Experiment 2, January 27, 1943.—A suspension of infected mouse lung in saline solution was used. Mrs. R. washed her hands with soap and water, rinsed them in running water, then with 95 per cent alcohol, and dried them under a sterile cloth for twenty minutes. Then the virus suspension, 0.2 c.c., was spread over an area of about 50 sq. cm. on the palm of the left hand, where it dried in twenty minutes. At the same time 0.2 c.c. of the same virus suspension was spread over a similar area in a Petri dish. After fifteen minutes in the dry state, each was resuspended in 0.4 c.c. saline solution and inoculated into mice. The four mice receiving material from the hand were killed on the twelfth day with the result being $12K_2$, $12K_1$, $12K_2$, and $12K_+$. The three mice receiving material from the dish were more seriously affected; one died on the fourth day with all lobes consolidated, another died on the twelfth day with extensive airless portions in all five lobes, and the third was killed on the twelfth day with all lobes involved ($4D_5$, $12D_5$, $12K_5$).

Experiment 3, February 18, 1943.—Allantoic virus from eggs was used, with a titer of 10^{-5}. Mrs. R. washed her hands with soap and water, rinsed in running water for five minutes, and then finally with 95 per cent alcohol. After the hands were entirely dry, the virus fluid, 0.2 c.c., was spread on the palm of the left hand where it became dry in fifteen minutes. After a further interval of ten minutes, the area was washed with 0.4 c.c. saline solution to resuspend the virus, and this material was inoculated into three mice. All died on the fourth day with consolidation in all five pulmonary lobes ($4D_5$, $4D_5$, $4D_5$). At the same time the allantoic fluid in portions of 0.2 c.c. was spread in each of two Petri dishes and allowed to dry. One of these portions was resuspended after being dry for ten minutes, and the other after being dry for 200 minutes. The mouse results were $4D_5$, $4D_5$, $5D_5$, and $4D_5$, $4D_5$, $4D_5$, respectively. An end point was not reached and apparently not even remotely approached.

Experiment 4, March 2, 1943.—Allantoic virus was used. Miss S. washed her hands with soap and water, rinsed in running water for five minutes, and then quickly with 95 per cent alcohol. After drying by evaporation, each palm was spread with 0.2 c.c. of the virus suspension. After drying seemed to have been complete for ten minutes, the material on the left palm was taken up in 0.4 c.c. of saline solution, and after forty minutes that on the right palm was resuspended in saline. The control on glass was made in the usual way and

taken up in saline solution after remaining dry for forty minutes. The results in the mice were as follows:

Left palm, dry ten minutes	$4D_5$, $4D_5$, $4D_5$
Right palm, dry forty minutes	$3D_5$, 5D no autopsy, $8D_5$
Glass, dry forty minutes	$3D_3$, $3D_5$, $5D_5$

Each entry shows, first, the day of death, second, D for natural death and K for killed, and, third, the number of pulmonary lobes involved. It is evident that the virus remained potent. As a control the normal allantoic fluid of embryonated eggs was introduced intranasally into three mice. These were killed on the tenth day and their lungs were normal.

Experiment 5, March 11, 1943.—Mrs. R. washed and dried her hands as she would habitually without any extra rinsing. Each palm was spread with 0.2 c.c. of allantoic virus, which dried after eight minutes. The same virus was also dried on glass. Mice inoculated in the usual way gave the following results:

Undried allantoic virus	$4D_5$, $6D_5$, $18K_2$
Dried on glass forty minutes	$4D_5$, $5D_5$, $6D_5$
Dried on hand ten minutes	$4D_5$, $5D_5$, $18K_0$
Dried on hand forty minutes	$5D_5$, $5D_5$, $5D_5$

One exceptional mouse appeared normal when killed on the eighteenth day ($18K_0$). However, it is evident that potent virus persisted in this experiment.

Experiment 6, March 17, 1943.—Mrs. R. washed her hands in the habitual manner and rinsed them with very soapy water which was allowed to dry. Then allantoic virus, 0.2 c.c., was spread on each palm and allowed to dry under protection of a sterile cloth. The usual control on glass was made. Mice inoculated as usual gave the following results:

Dried on glass forty minutes	$8D_5$, $8D_5$, $12K_1$
Dried on soapy hand ten minutes	$12K_0$, $12K_0$, $12K_0$
Dried on soapy hand forty minutes	$12K_0$, $12K_0$, $12K_0$

This result suggested that soap remaining on the skin might be a factor of significance in determining inactivation of the virus.

Experiment 7, April 27, 1943.—Mrs. R. washed her hands with soap and water, followed by rinse in running water for several minutes. Then one hand was given a final rinse with 95 per cent alcohol, and the other was finally rinsed in soapy water. The hands were then dried under a sterile towel for thirty minutes. Then allantoic virus of titer 10^{-2} was spread, 0.2 c.c. on each palm and also as usual on glass. The mice gave the following results:

Undried virus undiluted	$3D_5$, $5D_5$, $10D_4$, $11K_3$
Undried virus diluted 1:10	$5D_5$, $11K_1$, $11K_2$
Dried on glass ten minutes, undiluted	$5D_5$, $7D_5$, $8D_5$
Dried on glass ten minutes, diluted 1:10	$5D_5$, $10D_5$, $10D_5$
Dried on alcohol palm ten minutes	$11K_0$, $11K_0$, $11K_0$
Dried on soapy palm ten minutes	$11K_0$, $11K_0$, $11K_0$

Evidently the virus remained potent on the glass but not on the skin. Possibly the low titer of the virus used (10^{-2}) may have been a factor.

Experiment 8, May 4, 1943.—Mrs. R. washed her hands with soap and water and rinsed them in running water for several minutes. Then the left hand was given a final rinse with soapy water and the right hand was given a final rinse with 95 per cent alcohol. Both hands were held under a sterile cloth for thirty minutes, at the end of which time they were thoroughly dry. Allantoic virus, 0.2 c.c., was spread over about 50 sq. cm. on each palm and also on glass. The titer of the allantoic virus was found to be at least 10^{-3} in eggs. Results in the mice were as follows:

Undried virus	$3D_5$, $4D_5$, $6D_5$
Dried on glass thirty minutes	$3D_5$, $4D_5$, $6D_5$
Dried on soapy hand ten minutes	$10K_0$, $10K_0$, $10K_0$
Dried on alcohol hand thirty minutes	$10K_0$, $10K_5$, $10K_2$

Experiment 9, May 7, 1943.—Mrs. R. washed her hands and rinsed them thoroughly. The left hand then given a final rinse in soapy water and the right hand a final rinse in 95 per cent alcohol. The hands were dried under a sterile towel for thirty minutes. Allantoic virus of titer 10^{-5} was spread 0.2 c.c. on each palm and dried in seven minutes. After a further ten minutes the virus was taken up as usual in 0.4 c.c. saline solution. The usual control on glass was made. Results in the mice were as follows:

Undried virus undiluted	$5D_5$, $5D_5$, $10K_+$
Undried virus diluted 1:10	$4D_5$, $5D_5$, $6D_5$
Dried on glass ten minutes, undiluted	$3D_5$, $4D_5$, $6D_5$
Dried on glass ten minutes, diluted 1:10	$4D_5$, $5D_5$, $5D_5$
Dried on soapy hand ten minutes	$10K_0$, $10K_+$, $10K_0$
Dried on alcohol hand ten minutes	$5D_5$, $7D_5$, $7D_5$

Experiment 10, May 17, 1943.—Mrs. R. washed her hands as she would ordinarily and dried them on a paper towel. Each palm was then immediately spread with 0.2 c.c. allantoic virus. The left hand required seven minutes to dry and the right hand nine minutes. Controls on glass were made. Results in the mice were as follows:

Dried on glass thirteen minutes	$5D_5$, $5D_5$
Dried on glass forty minutes	$7D_5$, $10K_0$
Dried on right hand fourteen minutes	$10K_0$, $10K_0$, $10K_0$
Dried on left hand forty-five minutes	$10K_+$, $10K_0$, $10K_0$

Experiment 11, May 20, 1943.—Mrs. R. washed her hands as she would ordinarily and dried them on a paper towel. Then each palm was spread with allantoic virus 0.2 c.c. and both dried in ten minutes. The material was taken up from the right palm after a further ten minutes and from the left palm after forty-five minutes. The control on glass required forty-three minutes to dry and was taken up in 0.4 c.c. saline solution after a further 105 minutes. The original virus had a titer of 10^{-2} in eggs. Results in the mice were as follows:

Undried virus diluted 1:10	$4D_5$, $4D_5$
Dried on glass 105 minutes	$4D_5$, $4D_5$, $4D_5$
Dried on right hand ten minutes	$5D_5$, $8D_5$, $8D_5$
Dried on left hand forty-five minutes	$5D_5$, $7D_5$, $9D_5$

In this experiment there was persistence of the virus on the skin, even though it was an allantoic virus of rather low titer.

Experiment 12, May 29, 1943.—Miss L. washed her hands as she ordinarily would and dried them with a paper towel. Then 0.2 c.c. of allantoic virus was spread on each palm. Drying required eight minutes. The left palm was sampled after seventeen minutes and the right after fifty minutes. The control on glass dried in sixty-five minutes and was sampled after a further thirty-one minutes. Titration of the virus in embryonated eggs failed because of bacterial contamination. The results in the mice were as follows:

Dried on glass thirty-one minutes	$4D_5$, $4D_5$, $5D_5$
Dried on left hand seventeen minutes	$7D_5$, $9D_5$, $9D_5$
Dried on right hand fifty minutes	$9D_5$, $10K_3$, $10K_2$

DISCUSSION

Virus suspension made from the lungs of influenza mice used in the first two experiments seemed to be of rather low potency and to withstand drying very poorly. The lung virus used in Experiment 2 was perhaps slightly more active after drying on glass. This virus after drying on the palm of the hand also caused infection in all four inoculated mice, but of a milder type.

Experiment 3, in which allantoic virus was used, gave a strikingly different result in that all the inoculated mice died of the infection within five days and all with consolidation in all five pulmonary lobes. Here the virus evidently retained a high potency after being dry on the hand for ten minutes and after being dry on a glass surface for 200 minutes. In Experiment 4 the allantoic virus remained active forty minutes after it had been dried on the hand. In Experiment 5 the hand washed in the ordinary habitual way allowed the virus to retain its activity forty minutes after being dried on the skin. However, when soapy water was allowed to dry on the hands in Experiment 6, the virus subsequently dried on the same areas promptly lost its potency. An attempt to compare the inactivating effect of soapy skin with soap-free skin in Experiment 7 miscarried, possibly because of the low titer (10^{-2}) of the virus used. Repetition with slightly more potent (titer 10^{-3}) allantoic virus in Experiment 8 showed inactivation of the virus on the soapy skin in ten minutes and persistence of some virus for thirty minutes on the soap-free skin. Experiment 9, in which the allantoic virus had a titer of 10^{-5}, gave an even more clearly contrasted result, indicating loss of potency on the soapy skin and retained potency on the soap-free skin of the same person. The last three experiments 10, 11, and 12, show persistence of the virus on the hands washed in the ordinary manner and dried on a paper towel. Here the active virus could still be detected forty-five minutes and fifty minutes after drying had been completed.

Our results are somewhat at variance with those of Krueger and his associates. These observers used suspensions of mouse lung infected with influenza viruses of the P. R. 8 A strain and the Lee B strain. The hands were prepared by washing with soap and water and then held under a sterile towel to dry. Apparently special care to remove traces of soap was not exercised. Our observations were made with the Melbourne strain and for the most part

with virus-infected allantoic fluid, which seemed more potent than the suspensions of mouse lung which we used at first. Hence the technical methods were not identical.

The results of these drying experiments were somewhat variable and the variations cannot now be explained with entire satisfaction. However, the persistence of potency in an occasional specimen may be quite adequate to serve for transmission of the disease. We are therefore inclined to the view that soiled hands may reasonably be regarded as important for the spread of influenza, and especially the soiled hands of those concerned with the preparation and serving of food. Fortunately the virus of influenza appears to be readily inactivated by the ordinary soaps, a fact of importance to those who are concerned with the organization and inspection of establishments where food is served.

CONCLUSIONS

1. Potent influenza virus of the Melbourne strain may remain active on the palm of the human hand for forty-five minutes in the dry state.

2. The presence of soap solution tends to inactivate the virus on the skin.

3. Manual transmission of influenza, especially by food handlers, should be regarded as a possibility and a real menace.

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GIANT ORTHOCHROMATIC ERYTHROBLASTS*

Their Importance for the Promegaloblast and Pronormoblast Problem

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EVER since Cohnheim[1] observed large nucleated and nonnucleated hemoglobiniferous cells in the bone marrow of a fatal case of Addison-Biermer's disease, students of morphology have advocated the specificity of these erythroid elements (megaloblasts and megalocytes) for this particular disease. With the advent of intravital bone marrow studies many investigators reported megaloblasts in the normal adult human bone marrow. Segerdahl,[16] Sabin,[13] and Doan[3] are leading proponents of the hypothesis that megaloblasts are constituents of the normal bone marrow. The first worker suggests that the reticulum serves as the source of origin of this immature erythroblast, which, according to her, is morphologically identical with the promegaloblast of Addisonian pernicious anemia, whereas the latter investigators derive the cell from the endothelium of "intersinusoidal capillaries." Introzzi[5] stresses the origin of the megaloblast directly from the reticulum. Mollier[9] and Knoll[8] showed that the erythroblast (early pronormoblast) may develop directly from the reticulum under certain conditions. Downey[4] believes that normally the myeloblast of Naegeli[10] gives rise to the pronormoblast. Jones[6] adheres to the hypothesis that the pronormoblast as well as the promegaloblast of Addisonion pernicious anemia is derived from the myeloblast. Nordenson[11] proposes that both the pronormoblast and the megaloblast are derivatives of the reticulum. Rohr[12] favors the origin of the erythroblast from undifferentiated mesenchymal cells, thus being in accord with Naegeli's hypothesis. Rohr points out that the very young proerythroblast (early pronormoblast) is difficult to separate from the myeloblast. Dameshek and Valentine[2] state that "cells resembling the more mature megaloblasts are occasionally seen in conditions other than pernicious anemia." It is likely that the latter investigators observed giant orthochromatic erythroblasts.

In my own collection of normal human bone marrow obtained by sternal puncture the earliest recognizable erythroid cell "early pronormoblast" has a round reticular nucleus with its chromatin arranged similar to that of the myeloblast. The chromatin, however, is more coarse, and the parachromatin is thus more conspicuous as in the latter cell. The nucleus contains from one to several fairly distinct light blue nucleoli. A more or less well-developed perinuclear "Hof" encloses diffuse, fine-to-coarse granular oxyphilic protein, easily seen when the bone marrow is prepared and stained according to a method published elsewhere.[14] The cytoplasm is not abundant, generally showing from one to several cytoplasmic processes, and it ranges in color from light to medium blue. This early erythroid cell is derived directly from the reticulum and is

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morphologically neither identical with the promegaloblast nor is the cell difficult to distinguish from the myeloblast.

It is absolutely essential that the proper name be applied to early erythroid cells, because the type of erythroblast present in the bone marrow determines therapeutic measures, and a loose application of terminology must obviously lead to misinterpretation of the mechanism of normal and pathologic erythrogenesis. For example, Schulten[15] believes that the pronormoblast develops into a large hemoglobiniferous erythroblast (megaloblast) because of a lack of the antipernicious factor (hematopoietin). According to Sabin, Doan, Segerdahl, and others, megaloblasts increase in number because of the disturbance in the production or utilization of the antipernicious factor. Megaloblastosis is thus the expression of a maturation arrest. It is felt by most students of morphology that the megaloblasts, or any one of their later maturation stages, are neither constituents of the normal bone marrow nor are these cells products of faulty differentiation of already well-differentiated cells such as the early or late pronormoblast.

Megaloblasts are pathologic cells and indicative of a specifically altered physicochemical state of the reticulum. The early promegaloblast is derived directly from a diseased reticulum, and this mode of origin accounts for the characteristic morphologic features of the cell.

Certain workers believe that it is not possible to separate the early promegaloblast from the early pronormoblast and thus have adopted the size of the oxyphilic erythroblast as the criterion for separating Addison-Biermer's disease from other entities. The size of an erythroblast does not determine the diagnosis in spite of the fact that in Addison-Biermer's disease oxyphilic erythroblasts generally range in size from 12 to 27 microns. That giant orthochromatic erythroblasts are not specific to the bone marrow of cases suffering from extreme exacerbation of Addison-Biermer's disease will be shown below. Two cases are presented in which the aspirated sternal bone marrow contained numerous giant orthochromatic erythroblasts, although their etiologic factor differed. Thus the fallacy of using the size of an orthochromatic erythroblast as a pathognomonic criterion in the diagnosis of Addison-Biermer's disease is apparent.

It is the purpose of this paper to show how two bone marrow lesions were misinterpreted and therapeutic measures misguided through (a) lack of appreciation of the type of early erythroblasts present, (b) misconception of the variability and nature of one of the lesions, and (c) misuse of the term "megaloblast."

If megaloblasts are part of a given bone marrow pattern, their presence means only one thing: "Addison-Biermer's disease" (Addisonian pernicious anemia), or a biologic variation of this complex hereditary disease. Only by adhering to the fundamental morphology of the early erythroblasts one may hope to learn something about the pathologic physiology of diseases affecting the bone marrow organ.

Case 1.—A male, 79 years old, complained of extreme weakness and pain in the back on bending over. With the exception of an unexplained anemia, physical findings were essentially negative. The peripheral blood status revealed hemoglobin 48 per cent; red blood cells, 2,300,000; white blood cells, 4,000; mean corpuscular diameter, 8.4 microns. His private

physician performed a sternal aspiration and submitted the bone marrow smears to a pathologist for interpretation. Because of the presence of numerous "large orthochromatic erythroblasts" in the bone marrow, the lesion was interpreted as characteristic of Addisonian pernicious anemia. The patient received 10 units of liver extract daily for twelve days without benefit. The dose of liver extract was changed to 30 units twice weekly for five weeks. Patient failed to respond and was hospitalized in a moribund condition. Sternal bone marrow was obtained, and a study of the peripheral blood was made on the day of admission.

The bone marrow preparations showed numerous "giant orthochromatic erythroblasts" (Fig. 1*C*), which exceeded the early and late pronormoblasts (Fig. 1*A*) in number, the ratio being about 4 to 1. The diameter of these giant erythroblasts ranged from 15.8 to 39.6 microns. The cells appeared to have a normal quota of hemoglobin. The nuclei were either lobulated, fragmented, or consisted of from five to eight deeply staining masses of irregular size. Some of the nuclear masses were connected by fine chromatin strands. In the majority of the cells the nuclear masses were eccentrically located as shown in Fig. 1*C*. These giant erythroblasts could be traced to the reticulum by means of many intermediate cells. No cells were observed that showed the morphology of the megaloblasts of Addison-Biermer's disease. Many monocytoid reticulum cells were present either in small syncytia or as free cells which varied considerably in size. The morphologic patterns of the cells were distinctly pathologic. The bone marrow lesion was classified as belonging in the group of malignant lymphomata. Multipolar mitosis was not observed.

The peripheral blood showed: reticulocytes, 1.8 per cent; hemoglobin, 3.8 grams; red blood cells, 1,410,000; white blood cells, 3,200; hematocrit, 15 c.c. of packed red cells. For one hundred leucocytes there were eight oxyphilic erythroblasts ranging in size from 11.4 to 13.8 microns. Icteric index was 8 units. Gastric expression (alcohol-histamine test) showed no free hydrochloric acid. Examination of the bone marrow preparations made from the first sternal aspiration showed practically the same pattern as described above. Liver therapy thus did not alter the bone marrow lesion. Post-mortem examination revealed masses of various size in the mesentary. The tumors were made up of lymphoid tissue. Histologic diagnosis was "reticulum cell sarcoma."

Case 2.—A male, 82 years old, was admitted to the hospital in a moribund condition. Two months previously a sternal aspiration had been done by his private physician, and the bone marrow smears were submitted to a pathologist for interpretation. The striking feature in the preparations was the presence of a conspicuous number of very large "oxyphilic megaloblasts." Because the interpreter observed a lack of a diffuse basophilic megaloblastosis he believed the pattern to be the expression of some kind of malignant lesion (erythroleucemia). At that time the peripheral blood showed: reticulocytes, 0.3 per cent; hemoglobin, 5 grams; red blood cells, 1,560,000; white blood cells, 5,900. The blood smear showed a small number of erythroblasts in various stages of maturation. The patient received x-ray therapy over his slightly enlarged spleen. This treatment was reinforced by iron therapy. After the second x-ray treatment the patient failed to return to his physician. For three months previous to his hospitalization he was on a poor diet and without any kind of medication. On admission, a sternal aspiration was done. The preparations showed many "giant orthochromatic erythroblasts" (Fig. 1*D*) outnumbering the early and late basophilic erythroid cells in a ratio of about 3 to 1. The diameter of the giant erythroblasts ranged from 16 to 38.9 microns. The cells appeared to have a normal quota of hemoglobin. The majority of the giant cells showed a single round or oval nucleus. In others the nuclear mass consisted of three to seven more or less deeply staining nuclei eccentrically located. Thus the giant orthochromatic erythroblasts were morphologically identical to those observed in Case 1. An additional feature was the tendency toward multipolar mitosis in the early erythroblasts. Several giant orthochromatic erythroblasts showed mitotic figures. By means of many intermediate cells the giant orthochromatic erythroblasts could be traced to the reticulum. The early and late basophilic erythroid cells showed the characteristic nuclear pattern of the "megaloblasts" of Addison-Biermer's lesion of the bone marrow (Fig. 1*B*). In a recent paper by Jones,[7] the morphologic, physiologic, chemical, and biologic distinction of megaloblasts is reviewed in detail, and, therefore, a description of the megaloblasts is omitted. In addition, the myeloid cells showed the pathologic maturation pattern described

by Tempka and Braun[17] and Jones.[7a] The bone marrow lesion was classified as a unique variation of Addison-Biermer's disease. Examination of the peripheral blood showed: reticulocytes, 1.8 per cent; hemoglobin, 2.5 grams; red blood cells, 880,000; white blood cells, 1,900; mean corpuscular diameter, 9.5 microns; hematocrit, 12 c.c. of packed red cells; icteric index, 10. Gastric expression (histamine test) gave no free hydrochloric acid. The blood smear showed the characteristic pathology of the afore-mentioned disease. There were four oxyphilic erythroblasts with pyknotic nuclei among one hundred leucocytes. The erythroblasts ranged in size from 10.7 to 13.8 microns.

Patient expired a few hours after admission. Post-mortem examination revealed nothing remarkable. Weight of spleen was 240 grams. Final diagnosis was: advanced Addison-Biermer's disease (aplastic pernicious anemia).

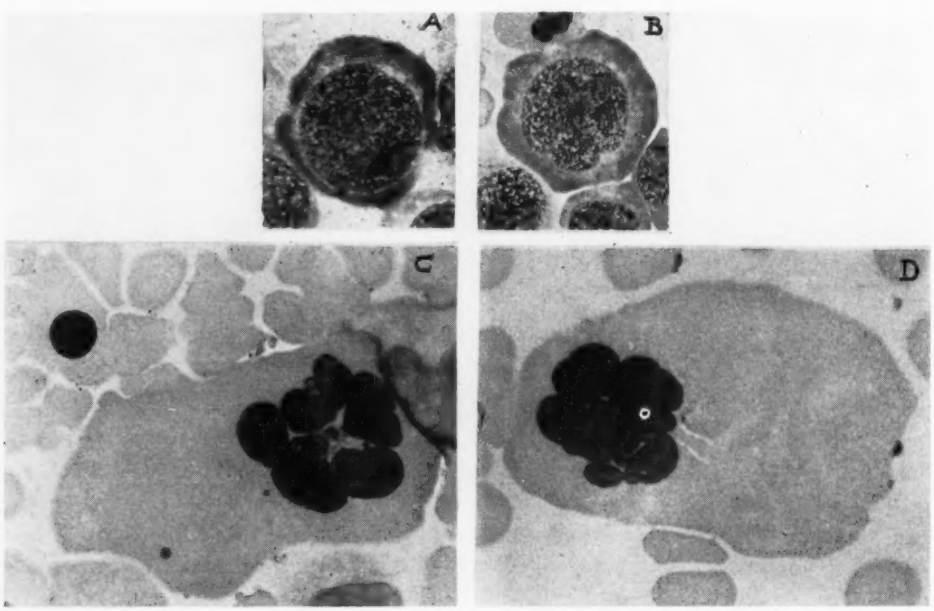

Fig. 1.—*A*, Early pronormoblast (Case 1). *B*, Early promegaloblast (Case 2). *C*, Giant orthochromatic erythroblast and orthochromatic macronormoblast in left upper corner (Case 1). *D*, Giant orthochromatic erythroblast (Case 2).

The histologic preparations of the post-mortem bone marrow showed several features of interest. There was a conspicuous number of Hedinger-Askanazy lymph follicles without germinal centers. Many of the collecting sinuses were markedly distended, thus giving an angiomatous appearance. The "Uferzellen" (reticulum cells lining the sinuses) were swollen and the nuclei were more or less pyknotic. Extensive reduction of hematogenic tissue and various degrees of sclerosis of the large- and medium-sized blood vessels as well as small areas of fibrosis, necrosis, and gelatinous transformation of fat were observed. In some of the sections made from material removed several centimeters away from the puncture, pools of blood were present, seemingly being the result of ruptured sinuses. Many megaloblastic islands were observed. Some tributary sinuses contained from a few to many polychromatic and orthochromatic giant erythroblasts, whereas the collecting sinuses contained only the small-sized oxyphilic erythroblast observed in the peripheral blood.

DISCUSSION

Intravital bone marrow studies are becoming more and more important in clinical hematology. The value of such studies, however, is proportional to the discrimination of the finer structural differences of the nuclei of the immature, that is, early bone marrow cells, by the examiner. Failure to appreciate these differences has led to much confusion with respect to the type of erythrogenesis present in the normal and pathologic human bone marrow. While on the surface the promegaloblast, pronormoblast, and giant orthochromatic erythroblast problem appears to be of academic interest, yet the type of erythrogenesis present in the bone marrow determines the therapeutic measures.

Generally it is true that the orthochromatic megaloblast of Addison-Biermer's disease exceeds in size the definitive oxyphilic macronormoblast, but it could be shown that neither large nor giant orthochromatic erythroblasts can be used as a means of separating Addison-Biermer's disease from other clinical entities, because both types may be produced in the bone marrow by diseases other than that just cited. Furthermore, the assumption that the early and late basophilic megaloblasts in untreated cases of Addison-Biermer's lesion dominate the polychromatic and oxyphilic erythroblasts does not hold in all cases. Thus it is easily seen that neither criterion is sufficiently constant to be diagnostic. Examination of several thousand bone marrow preparations representing a large range of diseases affecting the bone marrow organ revealed the fact that the most reliable criterion of the type of erythrogenesis present is the nuclear pattern of the early erythroblasts.

A comparative study of the first and second bone marrow biopsy of Case 1 showed that the liver therapy did not appreciably alter the bone marrow pattern.* Whether or not in Case 2 a timely administration of proper antianemic therapy would have had any beneficial effect on the pathologic state of the reticulum one may only guess. Attempted revitalization of a bone marrow that has undergone extreme physicochemical alteration complicated by irreversible lesions has met with failure in the few cases I have studied.

In both cases the early erythroblasts were derived from the reticulum, and, as can be clearly seen, each cell type shows a characteristic nucleus. The point of view of certain other investigators that the early promegaloblast and pronormoblast are not distinguishable is thus not valid. The giant orthochromatic erythroblasts were morphologically identical, but their precursors were different.

It is thus assumed that in either case the reticulum was differently affected because of the dissimilarity of the etiologic factor, leading on one hand to the production of pronormoblasts and, on the other, to the proliferation of promegaloblasts. It appears that a common mechanism was at work in the production of the giant orthochromatic erythroblasts, the nature of which, however, is conjectural. In none of the examined bone marrow preparations was I able to observe transitional stages between promegaloblasts and pronormoblasts, that is, the former giving rise or developing into the latter cells, or vice

*Dr. Hal Downey has in his collection a bone marrow from a case of leucemic reticuloendotheliosis that shows giant orthochromatic erythroblasts morphologically identical with those of Cases 1 and 2.

versa. This fact substantiates the opinion of those who believe that Addison-Biermer's disease affects the reticulum rather than the maturation of already well-differentiated cells.

It is assumed that normally reticulum cells go through an "inert" phase. During this latent stage one or more components of the hematopoietin exert their action upon the cells directing differentiation toward a specific cell type. Absence of one or more of these substances permits other factors to come into play, and such a faulty equilibrium between the bone marrow reticulum and the organs that control its normal function leads, for example, in Addison-Biermer's disease to the proliferation of promegaloblasts. The giant orthochromatic erythroblastosis is interpreted as the expression of a defunct reticulum.

SUMMARY

1. Two cases are reported which showed in the aspirated bone marrow numerous giant orthochromatic erythroblasts. Both cases were misdiagnosed because of a preponderance of these cells. Case 1 was afflicted with a reticulum cell sarcoma and Case 2 suffered from Addison-Biermer's disease (Addisonian pernicious anemia).

2. Giant orthochromatic erythroblastosis is not diagnostic of any specific disease. The phenomenon is the expression of a defunct reticulum.

3. The nuclear pattern of early erythroid cells, pronormoblasts and promegaloblasts respectively, is the only reliable means for judging the character of the erythrogenesis.

4. The early erythroblasts in either case were derived directly from the reticulum and showed characteristic nuclei.

5. The giant orthochromatic erythroblasts could be traced to the reticulum by means of intermediate cells. They were restricted to the bone marrow. Their morphology is described and illustrated.

I wish to thank Dr. G. E. Fahr, Head of the Department of Medicine, Minneapolis General Hospital, for his permission to publish the hematologic data of the cases presented here; Dr. Hal Downey for his kindness in permitting study of his case of leucemic reticulo-endotheliosis and for editing the manuscript; Dr. E. A. Sharp, Director of the Department of Clinical Investigation, Parke, Davis and Company, Detroit, Mich. for his interest in the anatomy, physiology, and pathology of the bone marrow organ. The photomicrographs were made by Mr. H. W. Morris, campus photographer.

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ADDENDUM

While this paper was in press, an article appeared in the Arch. Path. **36**: 127, 1943, by Limarzi, L. R., and Levinson, S. A., in which the presence of giant orthochromatic erythroblasts in the aspirated sternal marrow was reported. The patient apparently had a cancerous lesion of the bone marrow organ.

THE ANTIBACTERIAL EFFECTS OF VARIOUS ACRIDINE COMPOUNDS*

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FOLLOWING the demonstration of the antiseptic value of the acridine derivatives by Browning and Gilmour[1] in 1914, several of these compounds, namely, acriflavine and proflavine, were successfully used during World War I in the treatment of wound infections. Since that time, however, interest in such compounds has gradually diminished, and until quite recently their use for the treatment of wound infections has been largely abandoned. This lack of interest during recent years is perhaps explainable, in part, by the impetus given to studies of sulfonamide derivatives by Domagk, who demonstrated that striking therapeutic effects may be obtained in systemic infections following medication with the various sulfa drugs.

Although the sulfonamides have proved to be of unquestionable benefit in chemotherapy of many systemic bacterial infections, they have certain drawbacks as agents for rapid and effective healing of surface wounds.[2-5] The members of the acridine group which show antiseptic activity in high dilutions against the majority of pathogenic bacteria are also nontoxic in these same dilutions to mammalian tissues and do not suppress phagocytosis.[6] Furthermore, their activity is unaffected by the presence of serum or organic matter.[7]

In a previous report[8] from these laboratories there was submitted evidence of the comparative in vitro effects of several acridines upon gram-negative bacteria of the colon-typhoid-dysentery group. The present communication presents data on the in vitro effects of these and additional acridines upon *Staphylococcus aureus* ♯*209*, beta hemolytic streptococcus ♯C—203, *Streptococcus viridans*, *Streptococcus agalactiae*, Types I, II, and III pneumococci, *Bacillus pyocyaneus*, *Clostridium welchii* and *Vibrion septique*.

The compounds studied, together with their corresponding pH values in distilled water and broth, as determined by the glass-electrode potentiometer, are listed in Table I.

METHODS

A 1:1,000 dilution of each compound was prepared in a beef infusion 0.15 per cent dextrose broth of pH 7.4. A medium consisting of 1 per cent bactotryptone, 1 per cent neopeptone, and 0.15 per cent dextrose in distilled water and sealed under vaseline was used for the anaerobes. Serial dilutions through 1:512,000 were made in the same media. The tubes containing the drug dilutions and control broth tubes minus the drug were autoclaved at 10 pounds for 10 minutes. Each tube of a dilution series was then inoculated with a loopful of a 24-hour broth culture of one of the test organisms.

*From the Research Laboratories, Winthrop Chemical Company, Inc.
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TABLE I

NO.	COMPOUND	CHEMICAL FORMULA	pH OF 1:1,000 DILUTION	
			WATER	BROTH
1.	Acriflavine	3,6-diamino-acridine-10-methochloride-hydrochloride	5.0	7.4
2.	Proflavine	3,6-diamino-acridine-hydrochloride	2.5	7.4
3.	Rivanol lactate	2-ethoxy-6,9-diamino-acridine-lactate	5.6	7.4
4.	Rivanol-azo-sulfanilamide*	2-ethoxy-6,9-diamino-acridine-azo-sulfanilamide	4.5	7.4
5.	Rivanol-azo-sulfathiazole*	2-ethoxy-6,9-diamino-acridine-azo-sulfathiazole	3.5	7.4
6.	Atabrine dihydrochloride	3-chloro-7-methoxy-9(1-methyl-4-diethylamino-butylamino)-acridine-dihydrochloride	5.3	7.4
7.	Atabrine dilactate	3-chloro-7-methoxy-9(1-methyl-4-diethyl-amino-butylamino)-acridine-dilactate	6.2	7.4
8.	Atabrine dimethanesulfonate	3-chloro-7-methoxy-9-(1-methyl-4-diethyl-amino-butylamino)-acridine-dimethane-sulfonate	5.6	7.4
9.	Acranil	3-chloro-7-methoxy-9(2-hydroxy-3-diethylamino-propylamino)-acridine-dihydrochloride	5.2	7.4
10.	Neo-acranil	3-chloro-7-methoxy-9(2-hydroxyethyl-amino-ethylamino)-acridine-dihydro-chloride	5.1	7.4
11.	Entozon	2,3-dimethoxy-6-nitro-9(2-hydroxy-3-diethylamino-propylamino)-acridine-dihydrochloride	4.4	7.4

*The Rivanol-azo-sulfonamides were kindly supplied by Dr. Frederick Proescher, San Jose, California.

The tubes were incubated at 37° C. and examined for visible growth after 24, 48, and 72 hours. Lack of growth after 24 hours was considered evidence of bacteriostasis. Tubes which failed to show growth after 72 hours were tested for bactericidal activity by transferring 3 loopfuls of the drug-organism-broth mixture to a broth medium lacking the drug. Failure of growth to appear in the subculture tube was taken as evidence of bactericidal action on the part of the drug in the original medication tube. The results of these studies are presented in Tables II and III.

RESULTS

In summarizing the data given in the tables, we note that acriflavine, proflavine, rivanol lactate, and rivanol-azo-sulfanilamide give comparable bacteriostatic and bactericidal effects. Differences in the degree of effectiveness between any two of the above compounds upon the same test organism seldom involved more than two serial drug dilutions. Although the remaining acridines exhibited definite antibacterial properties, they were, in general, somewhat less effective than the first four compounds mentioned. The susceptibility of *Cl. welchii* and *V. septique* to the acridine compounds was of the same order as that of the gram-positive cocci. Much higher concentrations of the drugs were required, however, to inhibit the growth of *B. pyocyaneus*.

Unlike sulfonamide activity in vitro, which is generally recognized to be merely a bacteriostatic effect, the acridines used in this investigation exhibited a close correlation in the concentrations showing both bactericidal and bacteriostatic properties.

TABLE II

HIGHEST DILUTION OF COMPOUND SHOWING ANTIBACTERIAL ACTIVITY

COMPOUND	PNEUMOCOCCUS						STREPTOCOCCUS					
	TYPE I		TYPE II		TYPE III		HEMOLYTIC		VIRIDANS		AGALACTIAE	
	Bs	Bc	Bs	Bc	Bs	Bc	Bs	Bc	Bs	Bc	Bs	Bc
Acriflavine	128,000	128,000	256,000	32,000	128,000	64,000	256,000	128,000	64,000	32,000	256,000	256,000
Proflavine	256,000	128,000	256,000	128,000	128,000	128,000	256,000	128,000	128,000	64,000	128,000	128,000
Rivanol Lactate	64,000	64,000	128,000	128,000	128,000	128,000	128,000	64,000	64,000	64,000	128,000	64,000
Rivanol-azo-Sulfanilamide	128,000	64,000	256,000	128,000	64,000	32,000	128,000	64,000	64,000	64,000	128,000	64,000
Rivanol-azo-Sulfathiazole	16,000	16,000	64,000	32,000	8,000	4,000	16,000	16,000	8,000	4,000	16,000	8,000
Atabrine Dihydrochloride	128,000	128,000	128,000	128,000	64,000	64,000	64,000	32,000	16,000	4,000	16,000	16,000
Atabrine Dilactate	32,000	32,000	32,000	16,000	4,000	4,000	2,000	2,000	8,000	8,000	4,000	4,000
Atabrine Dimethane-sulfonate	32,000	32,000	32,000	16,000	4,000	2,000	2,000	2,000	8,000	8,000	8,000	4,000
Acranil	32,000	32,000	32,000	32,000	8,000	4,000	8,000	8,000	16,000	2,000	8,000	8,000
Neo-Acranil	32,000	16,000	32,000	32,000	8,000	4,000	16,000	16,000	16,000	2,000	8,000	8,000
Entozon	8,000	4,000	16,000	8,000	2,000	<1,000	16,000	8,000	4,000	2,000	16,000	8,000

Legend:—Bs = Bacteriostatic
Bc = Bactericidal

< 1,000 = Dilutions less than 1:1,000 not tested.

TABLE III

HIGHEST DILUTION OF COMPOUND SHOWING ANTIBACTERIAL ACTIVITY

COMPOUND	STAPHYLOCOCCUS AUREUS		BACILLUS PYOCYANEUS		CLOSTRIDIUM WELCHII		VIBRION SEPTIQUE	
	Bs*	Bc	Bs	Bc	Bs	Bc	Bs	Bc
Acriflavine	64,000	64,000	4,000	<1,000	256,000	8,000	256,000	128,000
Proflavine	64,000	16,000	4,000	<1,000	256,000	256,000	128,000	64,000
Rivanol Lactate	64,000	32,000	8,000	<1,000	256,000	4,000	128,000	64,000
Rivanol-azo-sulfanilamide	64,000	32,000	8,000	<1,000	64,000	32,000	64,000	16,000
Rivanol-azo-Sulfathiazole	8,000	4,000	2,000	<1,000	32,000	32,000	16,000	8,000
Atabrine Dihydrochloride	16,000	16,000	<1,000	<1,000	8,000	8,000	16,000	4,000
Atabrine Dilactate	8,000	4,000	<1,000	<1,000	32,000	16,000	8,000	4,000
Atabrine Dimethane-sulfonate	8,000	4,000	<1,000	<1,000	16,000	16,000	8,000	4,000
Aeranil	16,000	8,000	<1,000	<1,000	16,000	8,000	2,000	2,000
Neo-aeranil	16,000	4,000	<1,000	<1,000	2,000	2,000	2,000	2,000
Entozon	8,000	4,000	<1,000	<1,000	128,000	128,000	32,000	16,000

*See footnote under Table II.

CONCLUSIONS

Upon the basis of the experimental evidence presented in this report the following conclusions may be drawn.

1. Two acridine compounds, rivanol lactate and rivanol-azo-sulfanilamide, compare favorably in their antibacterial actions in vitro to acriflavine and proflavine.

2. Several atabrine salts and other acridine derivatives were found to exert definite bacteriostatic and bactericidal effects upon several of the organisms studied.

3. The mode of action of the acridines was found to be largely one of bacterial destruction rather than merely inhibition of growth.

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GENERAL CONSIDERATIONS ON PATHOGENESIS: SYPHILITIC AORTITIS, MYOCARDITIS, HEPATIC CIRRHOSIS*

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THIS paper presents a consideration of the possibility that allergic phenomena play a part in the development of certain diseases prevalent in Venezuela. Syphilitic aortitis, chronic myocarditis, and hepatic cirrhosis have certain clinical and pathologic features which provoke this discussion.

SYPHILITIC AORTITIS

The cycles of manifestations of syphilis are well known. In successive stages, the lesions increase in destructiveness, and in the tertiary stages, when the patient is sensitized and allergic, this is especially conspicuous. In Venezuela, as in other regions, syphilitic aortitis is more frequent than are gummas in other organs.

Syphilitic lesions were found in about 17 per cent of all autopsies and were the cause of death in more than 50 per cent of this group. The ratio of males to females was 4.9 to 1 as compared with 1.7 to 1 in the nonsyphilitic patients. This may be due to greater disposition of women to follow courses of treatment faithfully. In 500 cases of syphilitic aortitis, that lesion was the only morphologic evidence of syphilis in most of the cases. In less than 10 per cent of these cases were other organs involved. This material is especially suitable for study, because intimal arteriosclerosis is rare among the people of Venezuela and seldom offers a complication of the picture. Marked arteriosclerosis is not frequent even in the aged. In contrast, syphilitic aortitis is common and is frequently observed in young people. Indeed it is not rare for death to be due to perforation of an aortic aneurysm in patients in the early twenties.

Topographically, syphilitic aortitis presents certain peculiarities. The site of predilection is the first part of the ascending aorta. The process sometimes involves only the ascending aorta, but may extend to the abdominal portion. Frequently, in contrast to the appearance as noted in the study of the aorta in Europe, the line of demarcation between the diseased and normal parts is not abrupt but fades gradually. In other cases, the condition is limited to the abdominal portion, occasionally involving only a single isolated area. The gross characters of the lesions are similar to those usually described: roughening of the intima, with irregular, stellate or longitudinal puckerings, and elevated, opaque, white, porcelain-like plaques.

The histologic picture in general is that usually seen. Cellular infiltrations are present in the adventitia, particularly around the vasa vasorum, and accompany these vessels into the media, where larger accumulations of cells and foci of necrosis of muscle and elastic fibers often occur.

*From the Servicio Anatomo-Patologo del Hospital Vargas.
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Entirely independent of these more familiar lesions are the microscopic changes in the intima. These consist of patchy plaques of relatively acellular homogeneous material along the intimal surface. These plaques are almost colorless in hematoxylin-eosin stain, whereas with the van Gieson technique they are pink or somewhat more red in later stages. At these sites the thick wavy band of the internal elastic membrane is missing, thus forming gaps in the membrane. No elastic fibers are detected in these intimal lesions, unless there be newly formed fibers in old healed cases. In some instances, there may be an indistinctness or complete loss of the elastic fibers in the inner media.

The question arises as to how such different lesions have developed. I believe with Benda that the infiltrations in the adventitia and media are gummatous, produced by the effects of the spirochetes in situ, the actual presence of which in such lesions has been demonstrated in several instances. This explanation is not applicable, however, to the lesions of the intima, since inflammatory features are absent. That the reaction is a compensatory hyperplasia of the tissue seems unlikely, inasmuch as such plaques have been observed in the absence of any defect in the media. Rather, it seems to me that these intimal thickenings as well as the alterations of the elastic membrane are the result of an allergic process. Similar changes in the aortic intima have been observed in other allergic conditions.

The sequence of development may be outlined briefly as follows: During the acute syphilitic infection, with widespread invasion of the human body with spirochetes and with progressive multiplication and death of the organisms, the body becomes sensitized. During the subsequent stages, with the reactivation of the infection, new spirochetes in minor numbers lodge here and there in the wall of the aorta and set up an allergic reaction in the tissue.

The opinion has at times been expressed that, since the use of arsphenamine in the treatment of syphilis, the frequency of syphilitic aortitis has increased. Local syphilologists believe that the great number of cases of syphilitic aortitis in Venezuela may be the result chiefly of inadequate treatment and that such treatment is more dangerous than no treatment at all.

These beliefs are tenable in the light of my explanation of the genesis of the lesions. During the treatment with arsphenamine many spirochetes are killed in a brief period of time and thereby the human body is definitely sensitized. Some spirochetes, however, remain alive, though dormant. When reactivated, they excite an allergic reaction, and produce the features described above. Syphilitic aortitis must then be regarded as the result of a combination of the reactions caused locally by the reactivated spirochetes themselves and of the allergic reactions.

CHRONIC MYOCARDITIS

The term "myocarditis" is used here to denote only the condition of actual inflammation of the myocardium. Myocarditis has been observed remarkably frequently in the material at my disposal in Venezuela. It has been diagnosed in more than 15 per cent of the autopsies and has been the cause of death in two-thirds of these cases. About 450 cases of myocarditis have been proved histologically.

Numerous cases have presented the typical clinical picture of progressively developing insufficiency of the heart, leading to death within a few months, although the earliest symptoms had appeared one or two years previously. In striking contrast, other cases have occurred in apparently healthy young persons who suddenly dropped dead at their work or on the street. Such extremes in variation in the course of the disease are impressive. It is difficult to understand how persons with such serious cardiac damage have remained symptom-free, only to die so suddenly. From a careful investigation of 165 such cases, it is concluded that there is a causal relation between the myocarditis and the sudden death. In fact, in my series of cases, myocarditis has proved to be second only to perforated aortic aneurysms as the cause of sudden death.

The anatomic picture in all these cases of myocarditis is so uniform both macroscopically and microscopically that no morphologic classification is possible. The hearts in general are more or less dilated, often enormously dilated, without displaying a corresponding degree of hypertrophy. The dilation may affect the entire heart uniformly or may be greater on one side or the other. Only rarely, actual dilation is lacking and the heart is merely flabby or relaxed. In such instances the myocardium appears dull and "parboiled" and sometimes is spotted or streaked with smaller or larger scars.

Histologically, the changes vary greatly as to sites of involvement and slightly as to degree and quality, dependent upon the intensity and duration of the process. The cellular infiltrations consist chiefly of lymphocytes, sometimes plasma cells and large mononuclear cells, frequently eosinophilic leucocytes, but rarely polymorphonuclear neutrophils. These areas of infiltration are more or less circumscribed but are not sharply demarcated from the surrounding normal tissue. They vary in size and numbers and tend to accumulate in the subepicardial and subendocardial regions. Cases in which the cellular infiltrations are limited entirely to these regions have not been included in the group of myocarditis.

In other instances collagenous fibrous connective tissue is increased locally in larger and smaller areas. In these regions a certain number of muscle fibers are missing and have been replaced by young connective tissue, in which the same cellular infiltrations are present. Finally, there are extensive large scars. The entire muscle tissue may at times appear edematous and the muscle fibers cloudy; rarely, there are focal areas of finely granular and fatty degeneration. On the other hand, in some of these cases, the muscle fibers show fatty degeneration and cloudy swelling without any alterations in the interstitial tissue.

The lesions described are not specific, in no way suggest the etiology, and permit no classification into various subgroups. An attempt was made to correlate the myocarditis with other diseases. It was found that in 44 per cent of the cases syphilis was also present; in 48 per cent there was schistosomiasis; and in 28 per cent there was ankylostomiasis. Thus in some, namely 28 per cent, there was more than one disease. In our total autopsy material, these diseases appeared in 17, 18, and 7 per cent respectively. Thus the association with myocarditis is not merely a matter of chance. There is probably a causative relation. About 10 per cent of the cases of myocarditis were not associated with any of these three diseases. Most of them accompanied sepsis or other infectious diseases.

In order to gain some understanding as to the uniformity of the changes in chronic myocarditis associated with syphilis, schistosomiasis, and ankylostomiasis, a brief consideration of myocarditis as it occurs in other geographic areas is worth while.

Myocarditis has been observed in various infectious diseases. In the majority of these instances the organisms produce local alterations in the tissue. In others, however, as in diphtheria, the presence of organisms in the myocardium cannot be proved and the alterations must be in response to the action of toxins. In a large group of cases of myocarditis, called by various terms such as "isolated myocarditis" or "Fiedler's myocarditis," the cause remains uncertain. Purely chemical poisons, as illuminating gas (with its carbon monoxide), and several sulfonamides (French and Weller) produce alterations in the myocardium similar grossly and microscopically to the forms above described. In some parasitic diseases, as trichiniasis, myocardial changes are frequent, even when the parasites cannot be found in the myocardium.

In the well-known books and reviews (e.g., Mönckeberg; Saphir), attempts are made to subdivide the myocarditides on an etiologic basis. However, the etiology seems so uncertain that generally the authors speak only of myocarditis in typhoid fever, myocarditis in diphtheria, and so forth, suggesting that they assume a causal relation but do not wish to convey the impression that the localization of the organisms must be the cause of the focal changes. A classification of myocarditis from purely morphologic viewpoints is impossible. Classification in relation to accompanying infections is valuable clinically but is unsatisfactory pathogenetically. In the light of genesis only two great groups of myocarditis can be differentiated:

1. Those in which the organisms are actually in the myocardium and produce tissue reactions by their presence. Such cases include myocarditis of septicemia, tuberculous myocarditis, gummas of the myocardium, and several other forms.

2. Those in which the myocardium is free from the organisms but is indirectly influenced by the organisms at distant sites, as for example in diphtheria.

I shall now consider more in detail the myocarditis of syphilis, schistosomiasis, and ankylostomiasis.

There is no doubt that there are cases of severe syphilitic myocarditis with typical gummas, in which the spirochetes are present in the cardiac muscle. The literature reveals extensive controversy as to whether nonspecific myocarditis is related to syphilis proper. Warthin maintained that in men with latent syphilis, alterations of the myocardium can always be found; in women, they occur less often. By means of a special method he was able to demonstrate the presence of spirochetes in nearly all such cases. This result, however, was obtained only by Warthin and his pupils. Most other investigators have reported negative results. Saphir expressed the opinion that the bodies considered by Warthin to be spirochetes were artifacts and that he himself had been unable to find spirochetes in any of 130 cases of myocarditis associated with syphilitic aortitis.

I believe that the frequent association of myocarditis with syphilis, as evident in the material here in Venezuela, cannot be coincidental. The facts that neither typical histologic syphilitic characteristics nor the spirochetes

themselves can be found in such cases indicate that local growth of the spirochetes is not the underlying factor, that the changes are in no sense specific, but not that the alterations have no syphilitic basis.

The alterations of myocarditis associated with infectious diseases, in which no organisms are found in the myocardium, may be caused by products of the organisms growing in other parts of the body. This opinion, however, seems to me not to be applicable in the case of the myocarditis in syphilis, or if applicable, only to a very limited extent. It is well known that in the secondary stage of syphilis many spirochetes are distributed throughout the body but that these tend to disappear until spirochetes are found only in small numbers or not at all. Alterations caused by diffusible products should, therefore, be apparent in the secondary stage. I have had no personal opportunity to examine hearts from the secondary stage nor have I been able to find reports of such studies in the literature. Certainly all my cases are of the tertiary stage, with anatomic lesions of tertiary syphilis, namely syphilitic aortitis. In such cases, circumscribed lesions actually exist; these are associated with the local presence of spirochetes though in very limited numbers. It seems difficult, therefore, to attribute the lesions of myocarditis solely to the effect of diffusible products from the remote spirochetes.

It may rather be assumed that during the secondary stage of syphilis the body has become sensitized to the spirochetes circulating in the blood. During the reactivation of the process in the tertiary stage, the spirochetes concentrated in small numbers in certain other parts of the body are analogous to the "determining" injection in an animal experiment. We deal here with an allergic process.

The features in bilharzial myocarditis are similar. That lesions in the heart muscle are produced locally by the presence of the parasites or their ova must be rejected. The parasites live practically exclusively in the portal venous system. I found ova in the heart only once in 200 cases of schistosomiasis showing myocarditis. It is very probable that the parasites themselves secrete injurious agents.

Most individuals suffering from schistosomiasis are infected repeatedly. Consequently, here too the possibility exists that the injurious substances act directly upon the heart muscle and that an allergic state also exists, with a corresponding flare-up in reaction produced by a new infection. I would assume that even in the first infection the heart muscle is damaged, a fact which cannot be proved anatomically, although in the routine examination of hearts from persons with schistosomiasis a certain number exhibit more or less extensive fatty degeneration without true myocarditis. Experimentally, my former pupil, Romero Reveron, was able to show that in infected guinea pigs, alterations that could be proved electrocardiographically exist in the hearts, although histologically there were only small cellular infiltrations. Such findings suggest that the myocardium has been affected from the first. It seems probable that in the acute stage of schistosomiasis, the heart muscle is injured by the diffusible products; with repeated infections an allergic component is added, thus producing serious alterations in the previously injured organ. Experiments with repeated infections in guinea pigs are now in progress.

Similar conditions exist frequently in ankylostomiasis. Infections may occur, are cured, and recur after variable periods of freedom from the disease. In severe infections, cardiac muscle may be the seat of marked fatty degeneration. Thus, there occur (1) repeated infections with intervening periods of cure or latency, and (2) local damage to the organ during the original infections. This means that the conditions for allergic reactions are found. As a result of the different degrees of severity of the infection and the corresponding injurious effects, varying periods of latency, treatment, and so forth, many variations in tissue reactions are conceivable.

A further example of the same principle is observed in the chronic myocarditis of Chagas' disease. I have had only a few opportunities to study this condition histologically, since it occurs only in limited districts of Venezuela. Mazza has described and illustrated a large number of similar cases from Argentina. From the apparent histologic similarities to my own few cases, it seems to me that it would be impossible to make the diagnosis without knowledge of the clinical features and the detection of the parasites.

It is striking that in the acute stage of Chagas' infection also, a great many parasites circulate in the blood, reaching all organs of the body, whereas in the chronic stage, with chronic myocarditis, very few parasites are found in the myocardium. The situation of the parasites does not always correspond to the site or extent of the lesions. The question often arises as to how so few parasites may cause such extensive effects. My explanation is that in the acute stage when many parasites are present, the individual becomes sensitized, so that in the later chronic stage, when few parasites are present, there is an allergic reaction to even a small number. Mazza objected to my explanation of the genesis of myocarditis in general and suggested that all the cases of myocarditis which I have observed in Venezuela are manifestations of Chagas' infection. The similarity of histologic picture in the forms herein described justifies an attempt at explanation on the basis of allergy, common to all.

To summarize, the changes in the myocardium may be placed in two groups:

Group I, comprising the cases in which the infecting organisms live in the myocardium itself and cause alterations in the heart muscle as they do in other organs and tissues of the body.

Group II, comprising the cases of myocarditis in which the presence of the organisms in the myocardium cannot be proved. Such alterations can be found in association with bacterial diseases, parasitic diseases, or with purely chemical poisonings and metabolic disturbances. Hence, this group may be divided into two subgroups:

A. Comprising the cases caused by pure poisonings.

B. Comprising the cases in which the alterations depend upon allergic tissue reaction.

Frequently it may happen that both types occur in combination. Parenchymatous alterations probably appear first, followed by further changes as a result of the altered metabolism of the parenchyma. Clinically, such different pictures as insidiously and progressively developing chronic illness and sudden unexpected death are observed. It must be supposed that in the first instance, repeated slight allergic reactions aid the development of the entire process; in

the second instance, anaphylactic shock kills the individual suddenly. It is also understandable that no significant differences in the hearts are found clinically or anatomically, no matter what disease or process has caused the myocardial alterations. The toxic and allergic reactions of the heart muscle remain the same, irrespective of the original disease, and thus the pathogenic process remains the same in all cases.

HEPATIC CIRRHOSIS

Cirrhosis of the liver affords a particularly striking example of failure in classification by the purely analytic method. Undoubtedly, every author engaged in a detailed study of the cirrhoses has tried to produce a satisfactory grouping of these processes. A division according to anatomic features is not possible, since there are no finally distinctive characteristics. Even the most simple and common differences between the hypertrophic and atrophic forms are not fundamental differences. The same histologic picture can be observed in both forms, and sometimes it is quite impossible for the pathologist with only a histologic section at hand to determine whether the cirrhosis is of the hypertrophic or atrophic type. In fact, it seems that whether hepatic cirrhosis is hypertrophic or atrophic depends upon accidental, environmental factors or upon the stages of the process. Furthermore, attempts have been made to classify the cirrhoses according to its associated manifestations, as splenomegaly, ascites, icterus, etc. Studies of cirrhosis in schistosomiasis have demonstrated, however, that even such manifestations are highly variable, and I agree with the authors who reject such a method of classification. Moreover, if a classification is based upon etiology, there are two groups, hepatic cirrhosis of known cause and hepatic cirrhosis of unknown cause. The majority of cases would belong to the second group. Such a classification would not take into account the pathologic features and the pathogenesis. Another possibility in grouping would be on the basis of damage to the parenchyma or to the connective tissue.

This fundamental problem I have previously discussed, as have many other authors. In 1920, I described by means of repeated biopsies the course of cirrhosis of the liver, experimentally induced. I expressed the opinion that every substance which in large doses injures the liver parenchyma, in small doses repeated over a period of time is capable of producing cirrhosis and that cirrhosis is caused only by the substances that have an injurious effect upon the liver parenchyma itself. Recently I was able to prove that in bilharzial cirrhosis, one of the few forms of which the actual cause is known, this principle holds good. In rare cases of this disease with probable acute massive infection, I could find evidences of damage to the parenchyma, even extensive necrosis. In the chronic stages there were all possible forms of cirrhosis. Therefore, I regard bilharzial cirrhosis in man as comparable to an experimentally produced cirrhosis. I came to the conclusion that cirrhosis of the liver develops as a result of injury to the liver cells, whether these injuries are visible microscopically or not. Then the metabolic products of the damaged liver cells influence the surrounding tissue and cause the further changes, which in themselves are dependent upon the type and severity of the primary damage to the parenchyma, and upon the general condition of the reacting tissue or individ-

ual. According to Josselin de Jong, a great variety of agents may cause a great variety of anatomic pictures. The etiology, the intensity of the damaging influence, the portal of entry, the duration of the process, the age, sex, and resistance of the individual in question, constitution, racial characteristics, climate, and other factors included under the term of "environment" may play a role. In a recent publication I said, therefore: "All these factors appear to be significant, not so much with regard to the form of the cirrhosis as to the type and intensity of the parenchymal damage upon which the further changes are dependent." Later in the present paper I shall discuss how these processes may be explained.

In summary, it must be concluded that the conception of cirrhosis should be uniform and I agree fully with Fiessinger, who said: "The anatomic process of the cirrhoses is the same in all the cirrhoses; there exists only one cirrhosis."

The process, which brings about the various pictures in cirrhosis of the liver, is after all essentially similar to that described for myocarditis. There is, however, one difference, inasmuch as the process in myocarditis is initiated by bacterial or parasitic products, whereas the process in cirrhosis in the majority of cases is probably initiated by disturbances in metabolism. It is especially interesting to observe that such different causes can lead to essentially the same process.

Before going further into the fundamental problem of the pathogenesis, it is well to emphasize that I find no significant difference between diseases that lead to a diffuse parenchymatous injury of the liver and the forms of diffuse hepatic cirrhosis that represent the result of parenchymatous injury. We recognize as diseases with purely parenchymatous disturbances: first, catarrhal jaundice, and second, acute yellow atrophy of the liver. We now know that catarrhal jaundice represents a parenchymatous disease and that it is not always a mild and harmless malady that heals in a brief time and that there are severe forms that may lead to acute yellow atrophy. On the other hand, we know that acute yellow atrophy is initiated almost always by a condition that cannot be distinguished from catarrhal jaundice. It is probably correct, therefore, to assume that these two pathologic pictures represent only the extremes of a single disease complex, and that between the two extremes all possible variations can be observed. In some cases there is healing only after a long time; in others death occurs after a shorter or longer duration; yet other individuals suffer from repeated attacks, a condition that is termed recurrent yellow atrophy of the liver. In some instances post-mortem examination reveals a more or less typical picture of cirrhosis of the liver. Our present knowledge does not permit us to distinguish these features grossly and histologically from true primary cirrhosis of the liver, because such differentiation is possible only in the light of the clinical course of the disease. Therefore, it seems entirely logical, particularly if the above-mentioned conception of cirrhosis is accepted, to consider these two forms as cirrhosis. It would be unreasonable to exclude them, as did Askanazy, for instance, because fundamentally they cannot be differentiated from the other types of cirrhosis.

In cirrhosis of the liver, a direct stimulation of the connective tissue does not exist; the changes in this tissue must be regarded as of a "regenerative or

reparative kind'' (Rössle). The point emphasized by certain authors, for instance Rössle, that the reactions depend upon whether the damaging agents affect only the cells of the parenchyma or the connective tissue, Kupffer cells, or the capillary endothelium also, seems to me to be of minor importance. Injuries of this kind may be possible, although I have never observed them. They probably never occur without damage to the much more sensitive parenchymal cells. I believe, therefore, that I can justifiably present the following explanation of hepatic cirrhosis. It is a continuous process in which injury to the parenchymal cells is the first change. This stimulates regeneration and repair. Sometimes there is a compensatory regeneration of hepatic cells, but proliferation of connective tissue dominates the process. This explanation applies to all the conditions which belong in the broad field of hepatic cirrhosis and excludes from that category the purely toxic-degenerative, purely regenerative, and purely inflammatory lesions.

There is actually only one cirrhosis, and the various forms are caused mainly by variations in reaction. Such variations in reaction we denote today as allergy, and I believe that for the explanation of cirrhosis of the liver, allergic processes must be considered to a great extent.

Acute yellow atrophy of the liver is a sudden reaction of the entire organ to such an extreme degree that simultaneously an enormous number of cells die. Such a violent reaction could be explained only by acute and severe poisoning. It is known, however, that with only few exceptions, such as mushroom poisoning, no poison whatever can be detected. Experimentally, animals can be killed by agents that affect the parenchyma of the liver and serious alterations in the hepatic parenchyma can be produced without complete breakdown of the organ. In allergic processes violent reactions of the tissue occur, such as are seen in no other circumstances. Consequently, it seems logical to consider the process in acute yellow atrophy of the liver as an allergic reaction. Therefore, it is reasonable to believe that the liver has been affected by some previous damage and that with a new, even slight, injury an allergic reaction takes place in the previously diseased organ. If we start from this theoretical consideration, and if we accept the idea of the association between the damage of the parenchyma and the cirrhotic process, it would be logical to assume an allergic reaction also for the whole of the cirrhotic process. It then becomes clear that the reaction after a period of latency may be manifested in various forms, as a result of the intensity and duration of the injurious effect and may be dependent upon other external factors. Fundamentally, therefore, we must assume a damage to the liver parenchyma. Later, such damage may disappear or be reduced and allergic reactions may take place. Consequently, sometimes more toxic influences, sometimes more allergic reactions, and all possible combinations of these two factors may be the ultimate cause of lesions in the tissue. It is apparent that a great variety of possibilities leads to the diverse results and pathologic pictures.

These attempts at explanation are based upon hypothetical considerations. To me, however, these hypotheses have a logical foundation and facilitate a much better understanding of the cirrhotic process. Animal experiments were undertaken in 1930 to determine the validity of this hypothesis. Because of circumstances, these studies have not yet been completed, but give a clear

indication that allergic reactions play an important part in the development of acute injury to the hepatic parenchyma and the chronic lesions. Recently, Hartley and Lushbaugh came to similar conclusions.

SUMMARY AND CONCLUSIONS

An attempt has been made to show that often the analytic method cannot be employed to produce a really logical classification of diseases. In addition, it often fails to facilitate the understanding of pathogenesis. Clinical progress in diagnostic acumen and in therapy of diseases is possible only when the genesis of diseases is clear. For such understanding, I believe that synthesis is more important than analysis. We should not look for and describe the slightest variations in the alterations of the morphologic picture but we should rather search for the features in common, from which various deviations can be reasonably interpreted.

In studying the pathogenesis of various diseases, we must first know the etiology. Under etiology we must consider the extrinsic causative agent, as bacteria and parasites, or the intrinsic causative agent, as metabolic disturbances. However, different organisms and various disturbances in metabolism may both set up the same pathologic picture.

Equally important is the reaction of the body. It is a tenable assumption that the body may react similarly to various causes, especially if the etiologic agent affects the parenchymatous cells of organs and if these damaged cells produce further reactions as a result of faulty secretion and absorption of products of cellular disintegration. Furthermore, in many illnesses the pathologic processes may not be the result of a single transitory insult but often of a continuous or repeated insult. To these agents another important factor is added, namely, the allergic reaction of the individual, which in itself gives rise to the various tissue changes.

These considerations explain the fact that a single etiologic agent may produce diverse morphologic manifestations, and a variety of etiologic agents may operate to produce identical lesions. These deviations do not necessarily mean fundamental differences in the disease and separation into groups and subgroups, but simply indicate the manifold possibilities in the combined workings of the several factors.

To illustrate these principles, three entirely unrelated pathologic conditions have been considered:

1. In syphilitic aortitis, there is a combination of local tissue reaction to the organisms in situ and the allergic reaction.

2. Chronic myocarditis is to be regarded as a uniform process, in which there is primarily damage to the cardiac muscle fibers and, secondarily, mesenchymal alterations, with cellular infiltrations. This process, although purely inflammatory, must be distinguished from all myocardial inflammations in which organisms are present in situ. It is rather the allergic reaction of the cardiac muscle previously damaged. The myocarditis in syphilis, schistosomiasis, and ankylostomiasis have been shown to be of this type.

3. Although cirrhosis of the liver is caused by disturbances of metabolism in most instances, the same lesion can be produced by the injurious products of bilharzia. As a result of the findings in human beings and in animal experi-

ments, and by deduction, it is concluded that the process in cirrhosis of the liver is analogous to that in myocarditis; namely, that primarily there is parenchymatous damage and, secondarily, mesenchymal alteration. Cirrhosis, therefore, is the result of direct toxic effects combined with an allergic reaction.*

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*After having completed this paper, I found great interest in reading Sulzberger's book (Sulzberger, M. B.: Dermatologic Allergy: An Introduction in the Form of a Series of Lectures, Springfield, 1940, Thomas.) I was pleased to find that this author's ideas, as expressed in the last chapter, referring particularly to the question of the pathogenesis of syphilitic aortitis, tally with those presented in this paper.

THE PHARMACOLOGIC ACTION OF 2-METHYLAMINOHEPTANE (EA-1)*

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THE 2-methylaminoheptane is a clear, colorless, volatile fluid. It is only slightly soluble in water, but saturated aqueous solutions may be used for intravenous injections. The free base has the following formula:

$$CH_3—CH_2—CH_2—CH_2—CH_2—\underset{\displaystyle HNCH_3}{\underset{|}{CH}}—CH_3$$

Thus, the compound is an aliphatic amine, and it forms salts with a variety of acids of which I have generally used either the hydrochloride or the benzoate. Both are freely soluble in water, and a ten per cent solution has been used in the work described in this paper.† The hydrochloride and the sulfate are apparently not crystallizable salts, but the mucic acid salt is a fine white powder which lends itself readily to tablet manufacture.

In the experiments described herein this compound has been referred to as EA-1, followed by the designation of the salt used (HCl or benzoate as noted on the tracings). The EA-1 may be readily determined analytically by steam-distilling off the free base after previous addition of caustic soda to a sample and collecting the distillate in volumetric sulfuric acid and titrating back the excess acid with alkali, using methyl red as an indicator.

This compound belongs in the group of sympathomimetic amines, but its action differs from that of epinephrine in special features which will be discussed later. The EA-1 is a straight chain compound differing radically in its chemical structure from the usual sympathomimetic amines, such as epinephrine, ephedrine, neosynephrin, propadrine, benzedrine, paredrine, etc., all of which possess a benzene nucleus. Another straight chain compound which has been studied extensively is the nearly related 2-amino heptane,‡ which has been investigated especially by Proetz[1] and by Chen and their co-workers.

EA-1 produces notable symptoms when given by mouth in sufficient dosage; but the toxicity appears to be relatively low. For example, 3 c.c. of the 10 per cent hydrochloride solution (300 milligrams) given by means of a stomach tube to a dog weighing 24 pounds produced drooling of saliva, erection of a narrow streak of hair running in a central strip along the full length of the back, dilation of the pupils, slight lacrimation, rapid breathing, some depression, weakness, incoordination, and possibly some cardiac irregularity (missed beats?). Almost

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Since this paper was written the name "amethine" has been given to this drug.

†I have obtained these preparations from the Bilhuber-Knoll Corp. of Orange, New Jersey.

‡This drug has been made available by Eli Lilly and Company in the form of a 1 per cent solution of the 2-aminoheptane sulfate under the trade name of "Tuamine Sulfate", which is used as a vasoconstrictor in the nasal passages.

complete recovery occurred in two and one-half hours, but the pupils were apparently still somewhat dilated for some hours longer. Possibly the drug may exercise a similar mild but *prolonged* dilator action on the bronchioles, since the innervation is similar in both cases.

When given intravenously, for example 1 c.c. (100 mg.) of the hydrochloride, in 10 per cent solution to a dog weighing 14.5 pounds, symptoms similar to those described above were produced, but they came on somewhat more promptly and involved a more extensive group of structures. The heart was affected (in the main accelerated), the pupils dilated, the bristles were raised, salivation and slight lacrimation occurred, and mild nervousness and tremors were produced. The pupils were rather widely dilated, and the light of a lamp bulb flashed into the eyes caused only a moderate constriction. This moderate loss of the light reflex is apparently due to persistent stimulation of the sympathetic nerve endings in the radiating fibers, and probably not to weakening or paralysis of the sphincter muscle. Tremors and muscular weakness were more marked when the drug was given intravenously, but a dog of this size (14.5 pounds) may make a fairly good recovery in two or three hours from an intravenous dose of twice this size (i.e., up to 200 milligrams). But the dilation of the pupils seems to be rather persistent and may indicate a usefulness of the drug in this relation.

In one dog, however, in contrast to the symptoms noted above, I have observed a course of symptoms which I believe is worthy of special attention. In this animal on each of four occasions when the drug was administered, either by mouth or intravenously, the symptoms began and progressed practically in the same manner as described above for about one-half to three-quarters of an hour, but then symptoms of an entirely different character began to appear. There was some indication of nausea, anxiety, crouching, or creeping movements, some disorientation or slight disturbance of consciousness, and then quickly a full-fledged convulsive attack occurred, with complete loss of consciousness, vomiting, dilation of the pupils, frothing at the mouth, and tetanic involvement of the whole skeletal musculature with opisthotonus and complete incoordination. With two or three brief remissions, the whole attack would last four or five minutes. The animal then relaxed, but appeared in a dazed or semiconscious condition; the breathing being deep and rapid (probably largely from the asphyxia produced by the convulsions). In another four or five minutes, however, the animal began to recover rapidly, and in a little while consciousness returned; the animal got up, walked around, responded to petting, and soon appeared to be entirely out of danger, but the pupils remained dilated and the breathing was rapid, drooling of saliva continued, and the bristles were raised. These latter symptoms disappeared in the course of two or three hours and the animal again appeared to become perfectly normal. In short, this dog appears to get full-fledged, grand mal epileptic attacks each time the drug is administered. Apparently this animal is a chronic epileptic, and the attacks which occur during the course of the drug's action are merely superimposed on, or perhaps sandwiched in between, the various phenomena produced by the drug. I have not yet had an opportunity to administer the drug to other known epileptic dogs, but it is my impression that the drug will probably have a similar action if given in

large doses to other epileptic animals. Perhaps this observation should serve as a precautionary warning in regard to using the drug with epileptic patients. I hardly think that this *evanescent* epileptic-like type of convulsive seizure is specific with this drug in normal animals within the dosage range indicated above.

Fig. 1.—Carotid blood pressure in a dog with both brain and cord destroyed. At the left of the tracing the fall in blood pressure produced by pithing is shown. Following this ¼ c.c. of EA-1 benzoate solution (10 per cent) was injected intravenously and the resulting rise in pressure is shown.

In general, within therapeutic limits, this drug appears to have but very little direct action on any portion of the normal central nervous system, unless there may be some slight feeling of weakness, lethargy, prostration, etc., which possibly may be central in origin. Nausea or vomiting apparently do not ordinarily occur under moderate dosage of the drug.

Fig. 1 shows the action of EA-1 benzoate on the blood pressure in a dog that had been completely pithed, both brain and cord. After destruction of the brain and medulla an iron rod was passed down the full length of the spinal canal and left in place throughout the experiment. Complete destruction of the cord was verified by the presence of the rod in the canal at autopsy. Care was taken to make certain of this point because of special differences between the action of this compound and that of adrenalin. So far as the action on the blood pressure and circulation is concerned, the drug apparently acts exactly the same in the pithed animal as it does in the intact animal. It can be seen from this tracing that the blood pressure rise comes on fairly promptly but is much more persistent and stable than is that which follows adrenalin. Here the EA-1 greatly improved the condition of the animal and permanently raised the blood pressure. I have repeatedly saved animals when they were in a very low condition and about to die by injecting just the right-sized (*first*) dose of this drug. Apparently most other drugs may still exercise approximately their normal actions after the first dose of EA-1 has been injected. But this does not hold in all cases, for the first dose of EA-1, or of the nearly related 2-aminoheptane, or of other active derivatives of the heptane series produces changes in the animal which markedly alter the effects produced by the second or any later injections of any one of these particular compounds. Even members of this series which have but little blood pressure raising activity may, if injected first, prevent a full rise which normally would have been produced by a following (first) injection of EA-1 or of 2-aminoheptane. This tachyphylactic action is frequently quite marked with these drugs, and may be of importance clinically in some organs, although the possible extent of this action is now obscure.

Fig. 2 shows the action of EA-1 hydrochloride after large quantities of nicotine had been injected and the autonomic ganglia had presumably been paralyzed. It seems probable in this case that the EA-1 was a little more effective in raising the blood pressure after nicotine than it would have been had the nicotine not been given. It would seem that some sort of synergism here exists between these two drugs. And I have seen a number of other instances in which I suspected that EA-1 became more active after other sympathomimetic drugs had been given shortly preceding. The manner in which such synergistic actions may be effected can only be surmised at present. From the evidence presented in Figs. 1 and 2, it would be presumed that EA-1 must act in the main on structures lying peripheral to the autonomic ganglia. I have not, however, been able to determine with complete certainty whether or not there may, in the normal intact animal, be some action by the drug on the autonomic ganglia. It is my opinion, however, that the drug does not have any significant ganglionic action.

Fig. 3 shows the action of adrenalin and of EA-1 after ergotoxine ethanesulfonate. The ergotoxine was dissolved in a small quantity of ethyl alcohol, which was then diluted with water and injected intravenously. It is difficult to get the ergotoxine into satisfactory solution because a rather high percentage of alcohol must be present to prevent precipitation. And when such a mixture is injected into the blood it may be expected that much of the ergotoxine will be dropped out of solution. I have tried to inject enough of the ergotoxine (in

many small repeated doses) to bring about the reversal of adrenalin action. The first injection of adrenalin in Fig. 3 shows a small preliminary rise in blood pressure followed by a fairly typical fall. The preliminary small rise, I believe, was due partly to the volume of fluid (5 or 6 c.c.) used to wash the drug

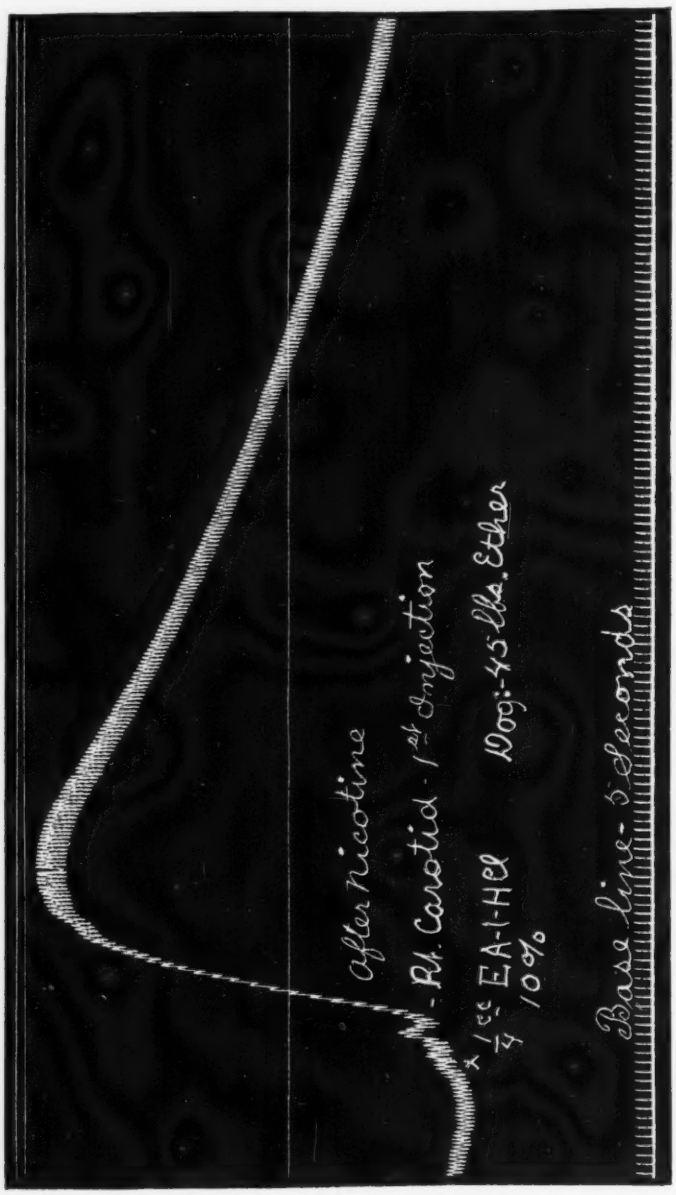

Fig. 2.—Carotid blood pressure tracing in a dog which had previously been given large quantities of nicotine by intravenous injection. The autonomic ganglia were presumably paralyzed by the nicotine.

into the vein and partly to a moderate acceleration of the heart beat from the action of the adrenalin. The curve of the fall and the recovery, however, seems to indicate that the vasoconstrictor endings no longer respond to stimulation by the adrenalin. Following this, an injection of ¼ c.c. of EA-1 hydrochloride solution (10 per cent) was injected, and a prompt and extensive rise in blood

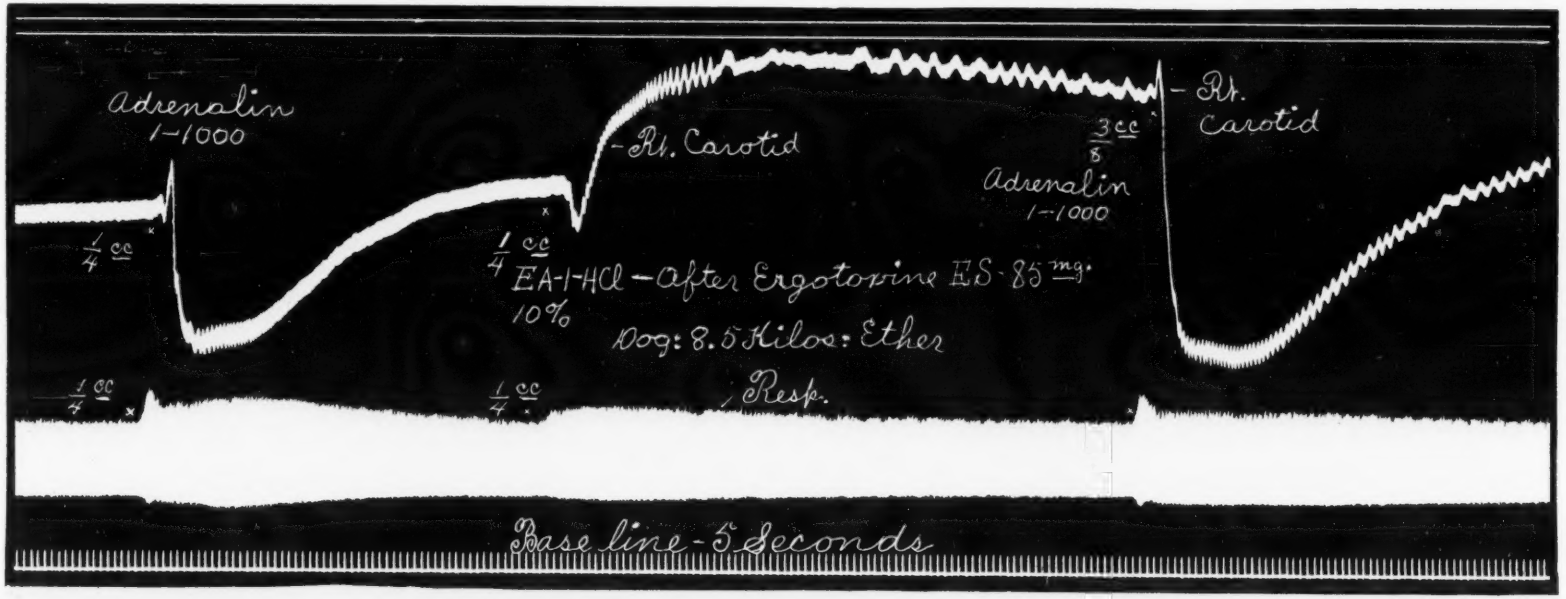

Fig. 3.—Carotid blood pressure and respiration. This animal had been given 85 milligrams of ergotoxine ethanesulfonate by vein, and at the left of this tracing ¼ c.c. of adrenalin solution was injected. This produced a fall (reversal) in blood pressure. After recovery, a dose (¼ c.c.)of EA-1 was injected and this caused practically a normal rise in pressure. A second injection of adrenalin produced a prompt fall, thus showing the difference in action of the two drugs after ergotoxine. For discussion, see text.

pressure was obtained. This shows that the action of this compound is different from that of adrenalin. And this point is further emphasized by the last injection (⅜ c.c. of adrenalin) shown in the tracing. Here a very marked reversal action by the adrenalin is virtually superimposed on the rise in blood pressure caused by the EA-1. I have repeated this experiment in a good number of dogs, and there can be no doubt but that the results shown here are very accurate. There is one point involved in this experiment which I should like to emphasize. I have regularly found that after repeated doses of ergotoxine have been given, injections of adrenalin will usually still produce fairly marked rises in blood pressure followed by only moderate falls with fairly rapid rise back to normal. If at that time a single small dose of EA-1 be injected it is found that thereafter adrenalin will produce a perfectly typical, and generally very marked,

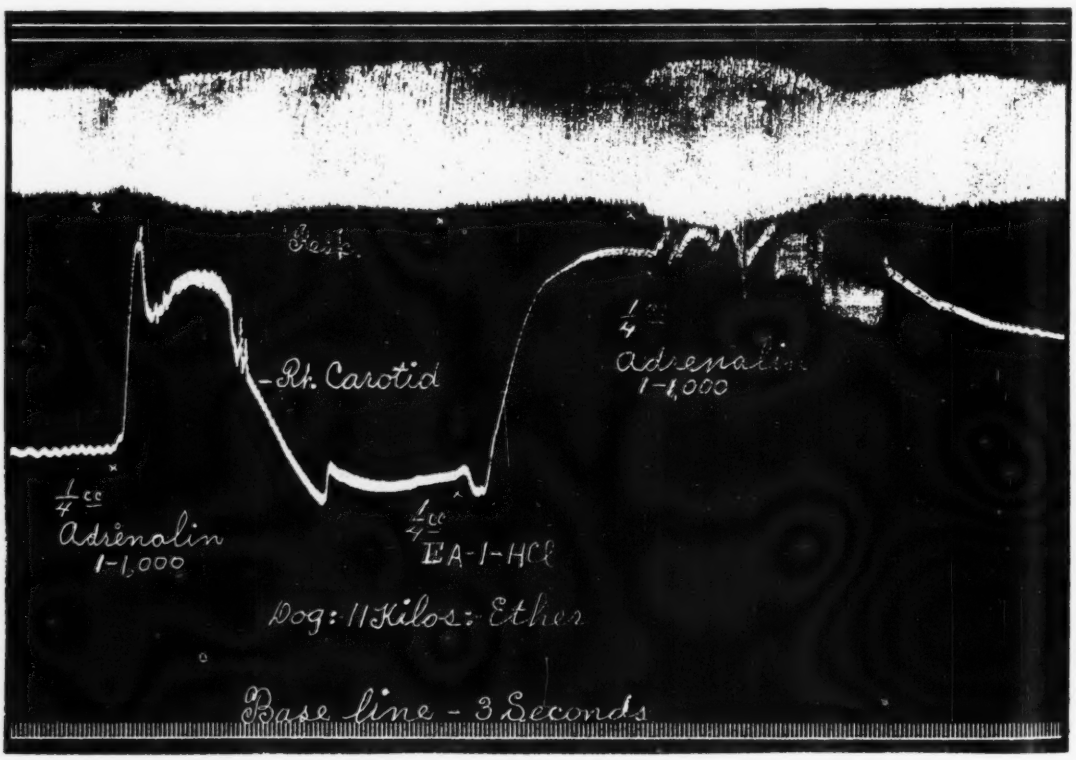

Fig. 4.—Respiration and blood pressure. This shows that the adrenalin action can be superimposed on that of EA-1. Note cardio-inhibition after adrenalin. For discussion, see text.

reversal fall each time the drug is injected. Thus the EA-1 appears to have some sort of synergistic action in the direction of making the reversal effects of the ergotoxine more prompt and emphatic, when adrenalin is injected later. Other sympathomimetic amines with which I have worked do not have this reversal action after ergotoxine and EA-1. But this phenomena led me to carry out the experiment illustrated in Fig. 4. In this experiment, as shown at the left of the tracing, a dose of adrenalin was injected and a typical adrenalin rise was produced. Following the return of the pressure to normal, a dose (¼ c.c.)

of EA-1 hydrochloride was injected. This produced a marked rise in pressure, at the highest part of which a second injection of adrenalin was given. The result is seen as a very obvious effort on the part of the circulatory system to raise the blood pressure still higher. The object of this experiment was to determine whether or not the EA-1 possessed any independent ergotoxine-like paralyzing action on the endings of the vasoconstrictor nerves. Apparently EA-1 merely acts synergistically with the ergotoxine but does not possess any independent reversing action of its own so far as adrenalin is concerned. I suspect this synergistic action with ergotoxine may be largely dependent on the cardiac action of EA-1 as illustrated in Fig. 5.

Fig. 5 shows the action of EA-1 on the heart and carotid blood pressure. Four separate injections were given, each ¼ c.c. Here the marked stimulation and improvement of the heart by the first injection is well shown. But the first dose does something to the heart which becomes very obvious following each of the succeeding injections. There are certain features shown here which I should like to emphasize. The first dose produces a very marked improvement in the strength and amplitude of the heart beat. The blood pressure in this animal was low at the start, but there is a progressive and well-sustained rise even though each new injection reduces the amplitude of the heart beat. I suspect that this changed reaction of the heart after the primary injection is the reason why EA-1 appears to increase the reversal action of ergotoxine to following injections of adrenalin. The EA-1, I suspect, has a double action on the heart; first a stimulation of the sympathetic nerve endings, and, second, a direct muscular action which is, with proper dosage, almost entirely stimulating and augmentative, but which rapidly changes to depression with the second or later doses. Even if the first dose be a little too large, there is quite likely to be a preliminary fall in blood pressure before the general rise begins. This preliminary fall is apparently due to a direct cardiac action. If one wishes to utilize the drug to stimulate and restore an animal which is about to die, then it is very important to inject the right-sized dose at the beginning, and generally any further injections are likely to be more harmful than beneficial. I have repeatedly seen such striking results follow this use of the drug that I have been inclined to suspect that it might be useful in severe cases of shock or in other conditions in which the blood pressure is very low. In these cases, only one dose should be given and it should be of the optimum size. In a rough way, by comparison in animal experiments, I have found a dose of ¼ c.c. of a 10 per cent solution of an EA-1 salt to equal approximately results produced by ¼ c.c. of the one to a thousand solution of adrenalin chloride. But the effects of the latter drug wear off much quicker than do those of EA-1. In Fig. 1 this type of improvement is seen in a dog in which the entire central nervous system is destroyed (and a considerable hemorrhage had occurred). In Fig. 6 this action is perhaps more strikingly illustrated. In this case the animal at the start was about to die; then artificial respiration was provided and two large injections of adrenalin were given. But in spite of all this, the animal almost certainly would have died. But when EA-1 (¼ c.c.) was injected, there was a prompt and sustained recovery of the heart and blood pressure. While most sympathomimetic drugs are not likely to be of much lasting help in cases of severe shock, yet I have rather strongly suspected that EA-1

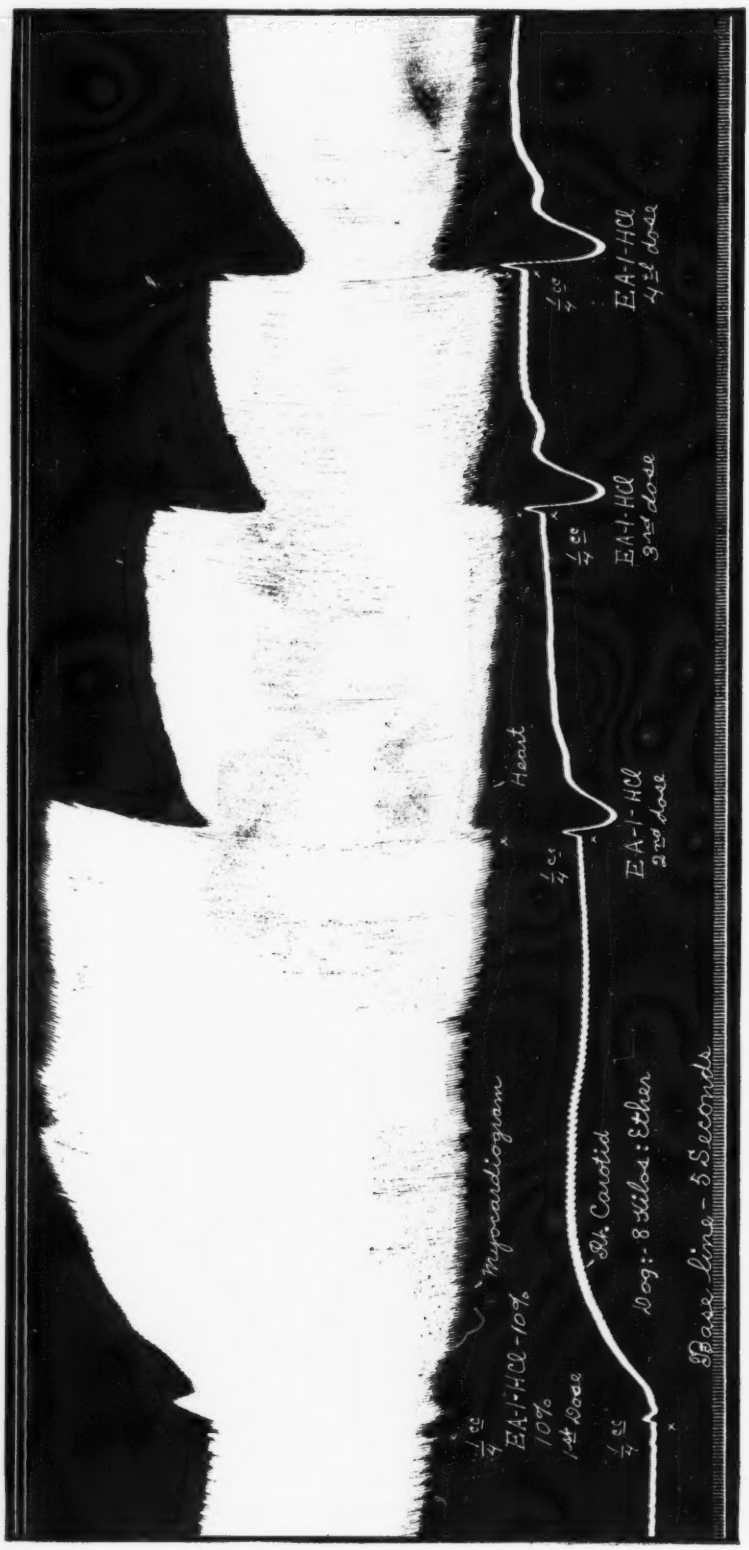

Fig. 3.—Myocardiogram and carotid blood pressure showing the results of four injections of EA-1. Note progressive rise in blood pressure.

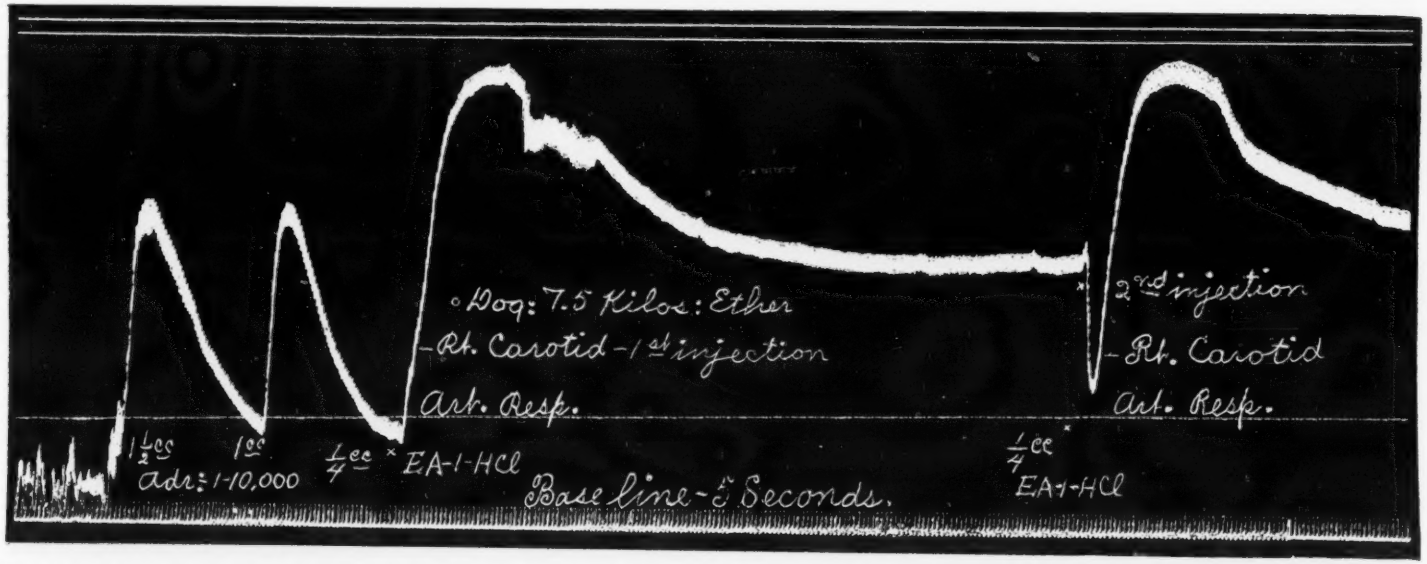

Fig. 6.—Carotid blood pressure in an animal near death. Showing comparative actions of adrenalin and EA-1. For discussion, see text.

properly administered intravenously might often tide a patient over until he could be brought to a place where more extensive treatment could be applied.

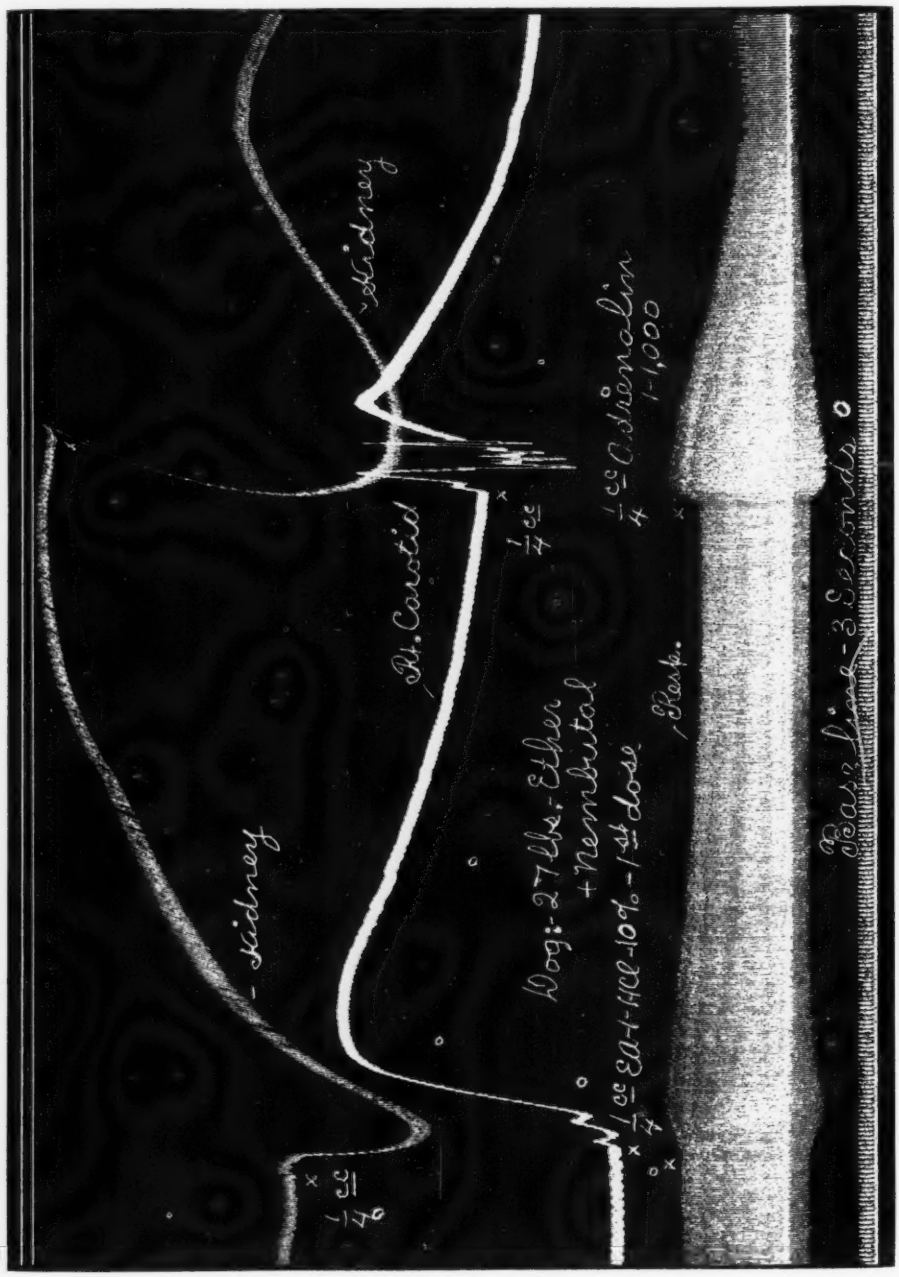

Fig. 7.—Kidney volume, blood pressure, and respiration showing the comparative actions of EA-1 and adrenalin. Note absence of cardio-inhibition after EA-1 and the marked inhibition after adrenalin.

One may readily see this action in an animal in which the brain, medulla, and upper part of the cord have been destroyed. And I have considered the possibility that this drug, if properly administered, might be used in some respects as a kind of substitute for digitalis. With moderate doses I have not seen as

much tendency to cardiac irregularities with EA-1 as would probably have occurred with comparable doses of the other sympathomimetic drugs with which I have worked. I have not made electrocardiographic records.

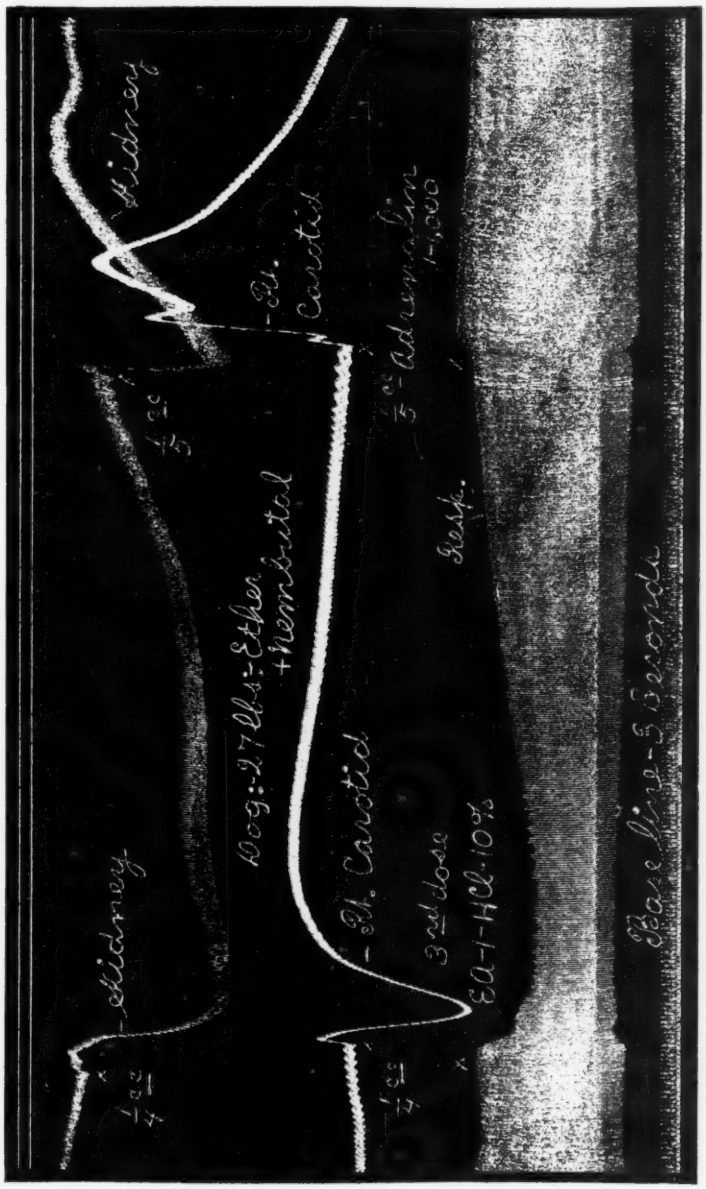

Fig. 8.—Kidney volume, blood pressure, and respiration showing comparative late effects of EA-1 and adrenalin.

Figs. 7 and 8 were both made in the same experiment. They show the results of the first and third injections of EA-1 together with injections of adrenalin for comparison. The action of EA-1 on the kidney is seen as a marked shrinkage in volume due to vasoconstriction. The vessels contract down to fully as small a caliber under EA-1 as they do under adrenalin, but the following dilation may vary somewhat in different experiments. Since the carotid

pressure in Fig. 7 rises much higher after EA-1 (the first dose) than it does in Fig. 8 (after the third dose) in which there is a considerable preliminary fall in pressure, one would expect that the kidney vessel constriction should be greater in Fig. 7 than in Fig. 8. This varies a good deal in different experiments, but in general it appears that the peripheral vascular constriction tends to become a little more intense with each repeated injection. This constriction is independent of the primary fall in pressure which tends to become more marked with each succeeding injection and which appears to be mainly due to the action on the heart. The vasoconstriction which occurs in the kidney is typical apparently of that which occurs in the other organs. The question might naturally arise as to what action EA-1 has on the arterioles of the pulmonary circulation, since these are in a somewhat different category from the systemic arterioles. Fig. 9 shows the action of EA-1 on the pulmonary blood pressure, which rises from fifty to one hundred per cent above the normal and tends to remain at this high level. The drug seems to have a very definite action here, and I believe that this is mainly due to stimulation and strengthening of the heart. There may be a specific constriction action on the pulmonary arterioles, but I believe that increased strength of the contractions of the right ventricle is the chief factor concerned. Fig. 9 also shows a profound constriction of the vessels in the nasal walls. It is to be noted that the drug here was injected into the femoral vein, but the vessels of the nasal walls and of the adjacent sinus membranes contract promptly just as do those of the kidney and other visceral organs. This is the usual action of sympathomimetic drugs, and it indicates a useful service which EA-1 may render in overcoming nasal congestion. The volatile free base may be inhaled for this purpose, or solutions of the salts may be applied locally. The nasal record (labeled 'nose') was made by closing off (airtight) the nasopharynx by means of a sponge rubber ball on the end of a rod, the ball being pressed against the soft palate which was thus forced backward against the posterior nasopharyngeal wall. Two glass cannulas were inserted (airtight) into the nostrils, and the cannulas were connected by means of a Y-tube and rubber tubing to a tambour. Shrinkage of the nasal walls (enlargement of the cavity) drew air out of the tambour, and the writing point moved downward on the drum.

Fig. 10 shows the action of EA-1 on the bronchioles. The animal was pithed and air was intermittently aspirated out of the chest in the usual manner with a plethysmograph placed on the chest. At the beginning of the tracing two injections (½ c.c. and 1 c.c.) of eserine solution were injected into the femoral vein in order to bring on a bronchoconstriction. When this became well marked, a dose (¼ c.c.) of EA-1 solution was injected. This caused a bronchodilation of moderate degree. A later dose of EA-1 did not produce any further dilatation. The effect of the EA-1 here is probably less than would have been produced by adrenalin. But the action may be more prolonged. There is also some likelihood that EA-1 may be effective on the bronchioles when taken by mouth. The action apparently depends on stimulation of the bronchodilator nerve endings. In passing, it might be noted here that electrical stimulation of the vagus nerves in the neck causes bronchoconstriction after EA-1 has been administered and also the heart is simultaneously inhibited, so that EA-1 does not paralyze

the ganglia. It might be noted in Fig. 10 that the blood pressure was very low (brain and medulla and upper cord destroyed) at the beginning of the record. But two doses of EA-1 brought the pressure up and maintained it at very nearly the normal level as it had existed before the animal was pithed. It would be rare that a patient would sustain a more severe injury than that involved in total destruction of the brain and upper cord (with the chest widely opened besides), so that the results produced here on the circulation might indicate some usefulness of the drug in severe traumatic shock.

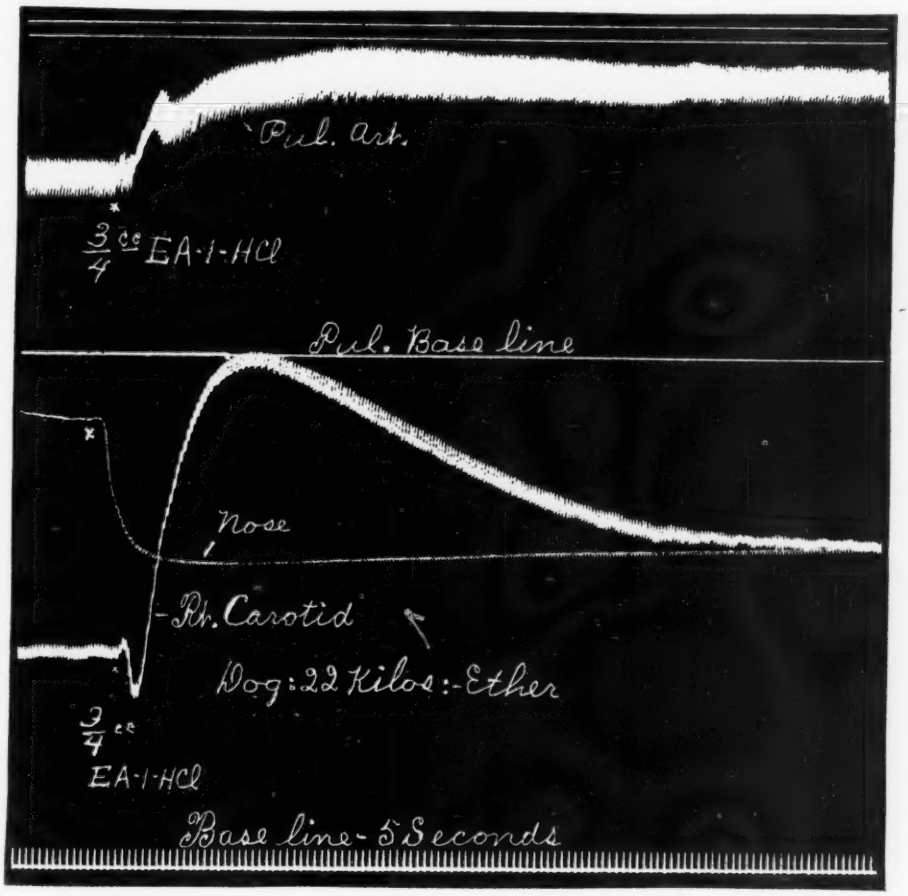

Fig. 9.—Pulmonary blood pressure, nasal cavity volume ("nose") and carotid blood pressure showing the action of EA-1. Note absence of cardiac inhibition. For discussion, see text.

In connection with the respiration, it might be noted that in anesthetized animals large doses of EA-1 cause death by paralysis of the respiratory center. But there is seldom seen any respiratory stimulation in animals anesthetized with ether or nembutal.

The usual action on the stomach in anesthetized animals is shown in Fig. 11. Some relaxation and inhibition of tone is obvious, but the action is less pronounced than that produced by adrenalin as shown in the right hand portion of the tracing. But the action of EA-1 is more lasting, and in addition it probably

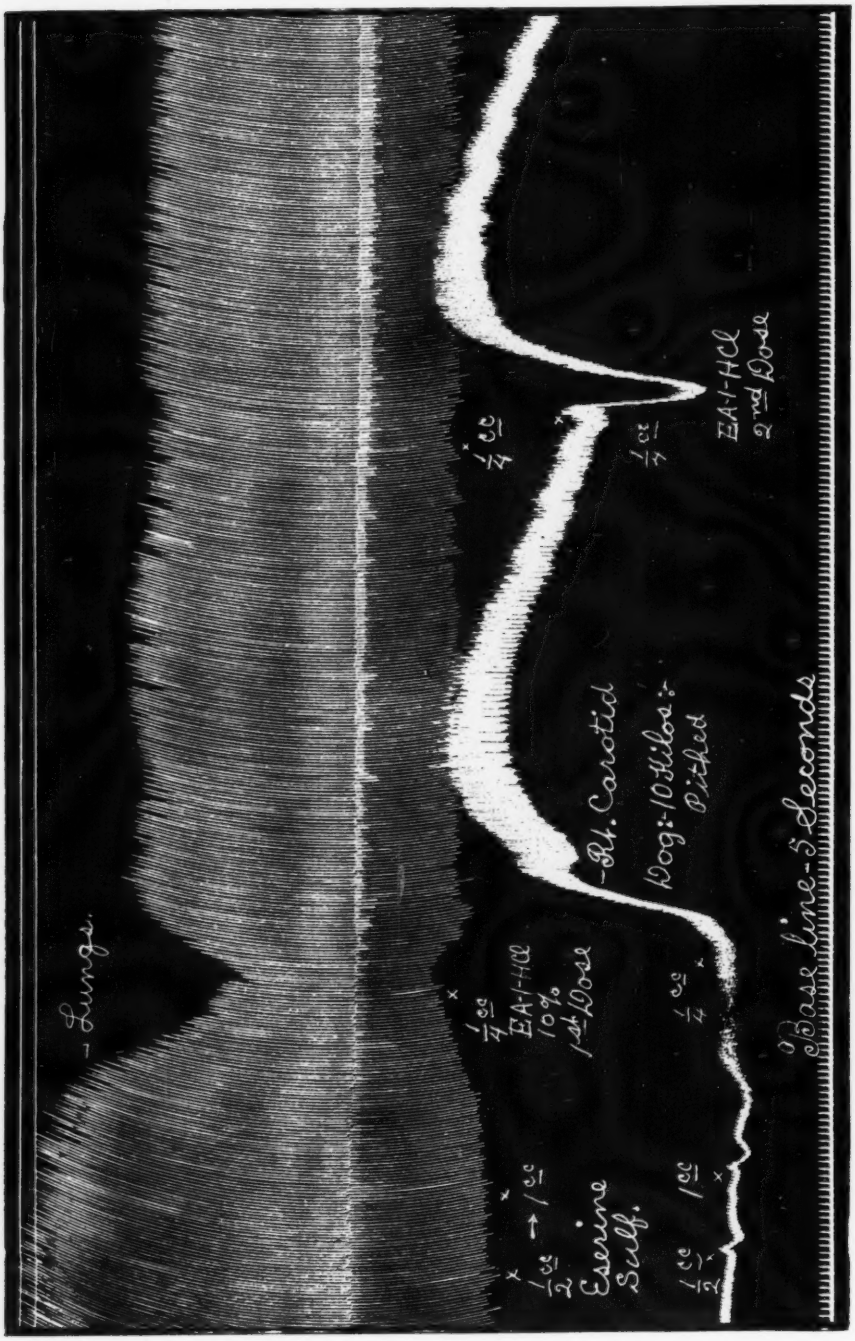

Fig. 10.—Lung tracing and carotid blood pressure in a pithed dog. Showing the bronchodilator action of EA-1 after eserine had produced constriction. See text.

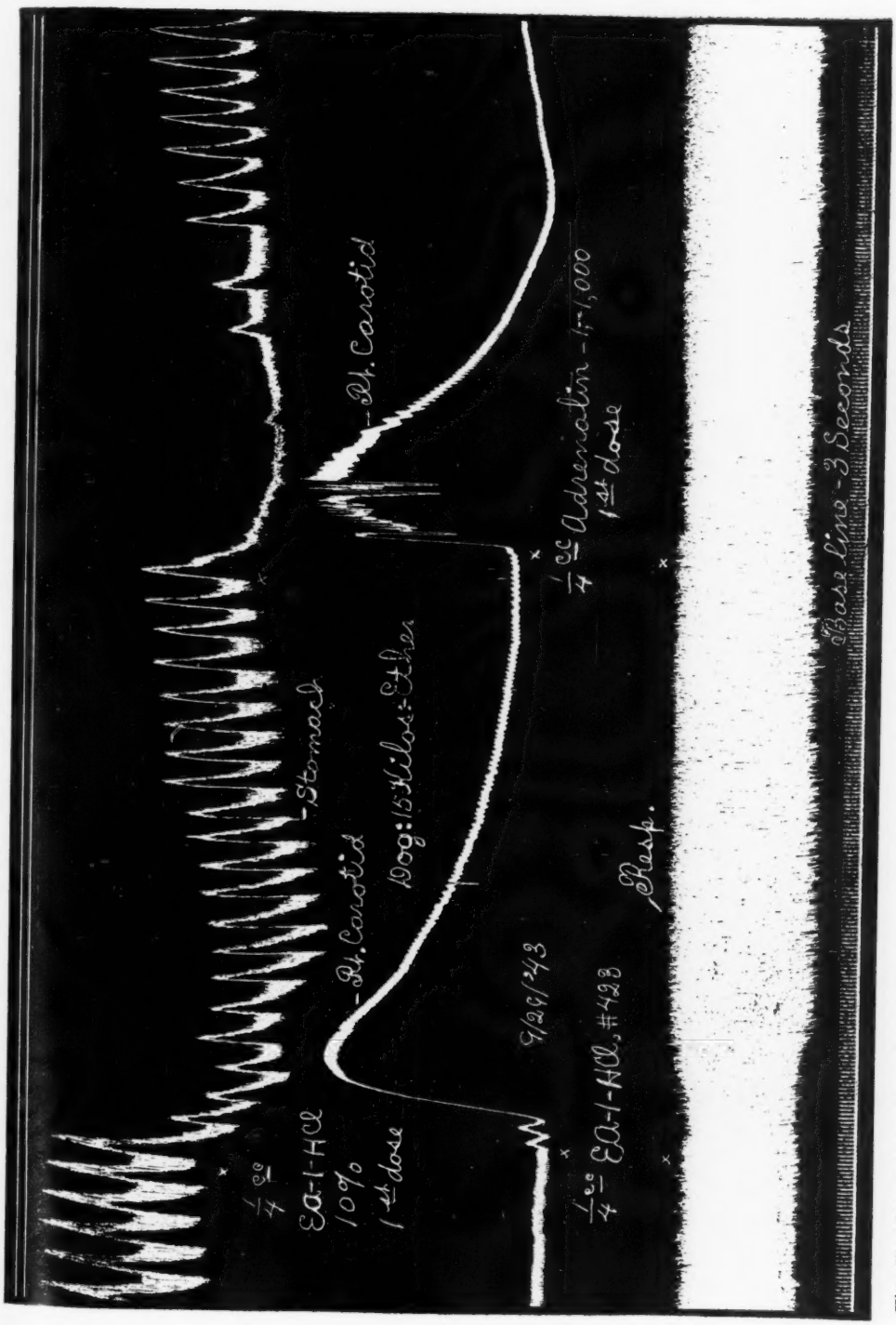

Fig. 11.—Stomach tracing, blood pressure, and respiration. Showing the comparative actions of EA-1 and adrenalin. Note cardiac inhibition after adrenalin but not after EA-1. The pressure elevation was approximately the same in each case. See text.

can be elicited by oral administration of the drug. Also the intestines are not much affected by EA-1 in acute experiments. It seems that the inhibitory action of the drug is, in general, considerably less marked than is the motor action on the circulation. And the bladder is also but little, if any, affected. It is difficult to surmise what kind of clinical effects might be obtained by an action on the stomach such as that shown in Fig. 11. Would such a relaxation in tone bring relief from colicky pains even though the peristaltic movements continued?

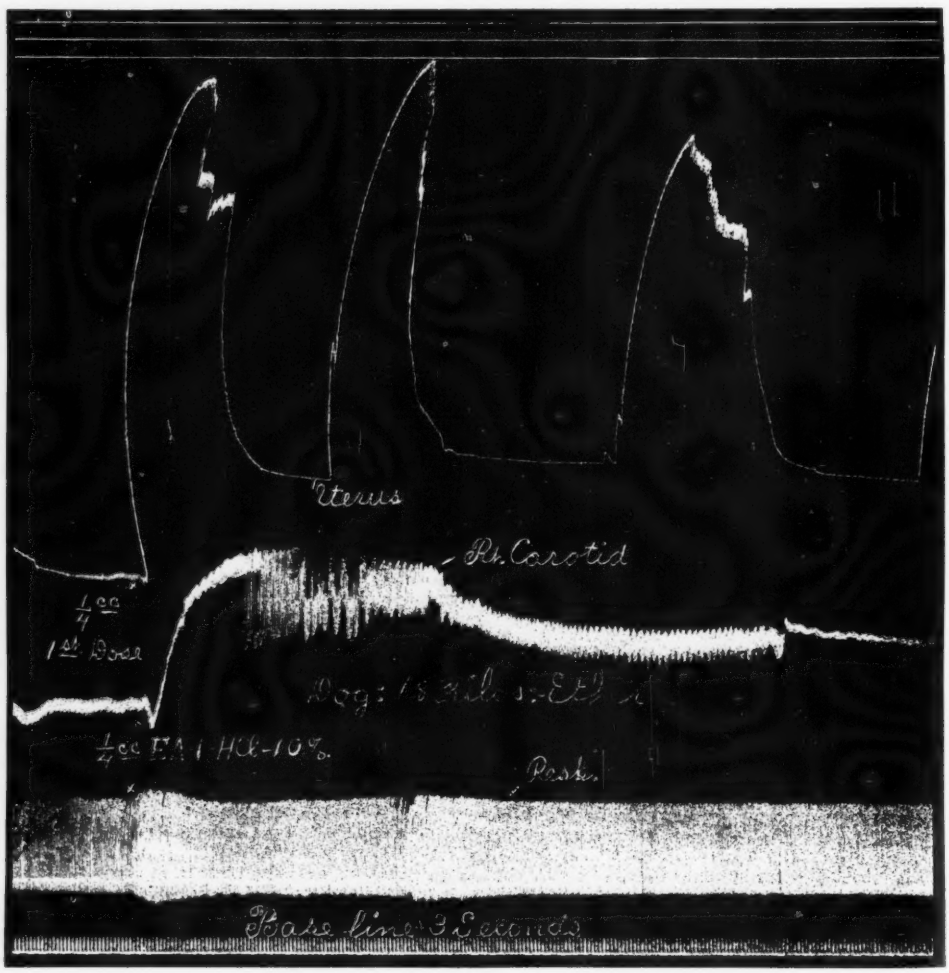

Fig. 12.—Uterus tracing, blood pressure, and respiration in an animal near term. Note cardiac inhibition (rare) in this case after EA-1. For discussion, see text.

It seems that EA-1 has but little, if any, action on the uterus. Fig. 12 shows a series of vigorous uterine contractions in an animal near term. The injection of EA-1 produced a marked action on the circulation but so far as the uterus is concerned only one thing can be said, viz., that the contractions were not inhibited. There may possibly be some slight increase in the general tone level of the uterine tracing (intact animal). But the marked intermittent con-

tractions shown in the tracing were occurring spontaneously before the drug was injected, so that the only conclusion permissible is that EA-1 does not inhibit the contractions. That point might be significant in case the drug should be used during labor. In other experiments in intact animals I have failed to note any special action of any kind on the uterus.

I have presumed that the chief action of this drug on the circulation involves primarily the heart and arterioles. But the capillaries may also be especially involved, by an action somewhat the reverse of that of histamine. The drug bears some similarity to ergotoxine in its action, and this has led me to wonder if it might be useful in migraine. I hardly believe that its continued use would lead to gangrene. I have no suggestions regarding any relation which the action of this drug may have to adrenergic, cholinergic, esterase, hormonal, etc., phenomena. But it would be very interesting to study this compound in accordance with the splendid method of clinical assay of sympathomimetic drugs which has recently been so well developed by Vaughan, Perkins, and Derbes.[2]

A consideration of the action of a compound such as EA-1 calls to mind such subjects as asthma, angioneurotic edema, anaphylactic shock, urticaria, ciliary activity, general allergic conditions, nasal decogestion, local vasoconstriction on hypodermic injection, sympathetic secretions, traumatic shock, pupillary dilatation, cardiac stimulation, serum sickness, development of tolerance for the drug, etc.

SUMMARY

The general pharmacologic action of a special sympathomimetic drug, 2-methylaminoheptane (EA-1), is discussed and illustrated by tracings.

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1. Proetz, A. W.: 2-Amino-Heptane Sulfate as a Nasal Vasoconstrictor, Ann. Otol., Rhin. & Laryng. **51**: 112, 1942.
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SIMPLIFICATION OF THE TREATMENT OF DIABETES*

Capt. Lazarus L. Pennock, M.C., U. S. Army

TODAY, more than twenty years after the introduction of insulin in the treatment of diabetes, the prescribing of diabetic diets is still in a state of mild confusion. It seems that every writer on diabetes has his own method for writing a diet. As a result, many methods for writing diabetic diets have been described. Some use complicated surface area tables. Others use set standard formulas, four to six of them, for all diabetic patients. Even a slide rule has been designed, to calculate a diabetic diet, by a drug firm. And in competition another firm has brought forth a "simpler" mechanical aid for the same purpose.

All this has made for confusion among the average practitioners of medicine who see a few diabetic patients now and then. In despair, most of the diabetic patients are referred to a specialist. A simple but satisfactory formula for computing a diabetic diet has been a necessity for some time. Such a diet should be individualized, tailor-made for each patient, standardized according to physiologic principles to meet the needs of each patient. And it should be properly balanced according to the best of our knowledge of nutrition today.

The essential thing to be borne in mind is that the diabetic diet should be a physiologic one, based on known values of the normal diet. The average individual, left to himself, chooses a diet that is not physiologic. He eats too much and the components of his diet are not proportioned in accord with what has been proved to be ideal for optimum nutrition. This is the chief fallacy of the so-called "free diet" treatment of diabetes.

The dietary treatment of diabetes is no longer, and in point of fact never was, a question of high carbohydrate versus high fat diet. Since a physiologic diet is to be prescribed, examination of such a diet reveals that the total daily caloric allowance is divided into carbohydrate, 65 per cent; protein, 15 per cent; and fat, 20 per cent. Such an arrangement always yields a diet that is high in carbohydrate and proportionately lower in fat. Hence, the diabetic diet should approach these proportions as nearly as possible, and the method of calculating a diet that will be described here does just that in a very simple manner.

In addition, the diet is calculated for the patient's normal or physiologic weight. Such weights are derived from the actuarial tables for age-height-weight, one of which is presented herein and which may also be found in almost any book on diabetes or nutrition.

These facts constitute the background for the method to be described. The method has been in use since 1924, when it was first propounded by Barach. For the past eleven years I have not only seen it applied, but have used it myself and watched it work successfully in all types of diabetic pa-

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tients. It never fails if one keeps in mind that, once the diet has been prescribed, the patient should be maintained on it until his metabolism has become adjusted to the diet. This will invariably come about in the course of a few days. Then, if the patient still continues to show sugar, insulin is given in sufficient amounts to control the hyperglycemia and glycosuria without changing the diet as a whole.

There are two essentials in calculating a maintenance diabetic diet. They are (1) determining the total daily caloric allowance per day for the patient and (2) dividing this allowance into the proper proportions of carbohydrate, protein and fat.

STEPS IN CALCULATING A MAINTENANCE DIET

1. Determine normal body weight in pounds for age and height of the patient (Table I). Convert pounds to kilograms by dividing by 2.2 (Table II).
2. Determine the caloric allowance per kilogram normal body weight.
3. Multiply kilograms normal weight by caloric allowance per kilogram for total daily caloric allowance.
4. Divide the caloric allowance into carbohydrate, protein, and fat.

TABLE I

NORMAL WEIGHT—17 TO 34 YEARS

AGES	17—19	20—24	25—29	30—34
HEIGHT	M—F	M—F	M—F	M—F
4′ 10″	109— 1	115— 4	120— 6	123— 6
4′ 11″	111— 1	117— 4	122— 6	125— 6
5′ 0″	113— 1	119— 4	124— 6	127— 6
5′ 1″	115— 1	121— 4	126— 6	129— 6
5′ 2″	118— 1	124— 4	128— 6	131— 6
5′ 3″	121— 1	127— 4	131— 5	134— 6
5′ 4″	124— 1	131— 5	134— 5	137— 5
5′ 5″	128— 2	135— 6	138— 6	141— 5
5′ 6″	132— 2	139— 6	142— 6	145— 5
5′ 7″	136— 2	142— 5	146— 6	149— 5
5′ 8″	140— 2	146— 5	150— 6	154— 6
5′ 9″	144— 3	150— 5	154— 6	158— 6
5′ 10″	148— 3	154— 5	158— 6	163— 8
5′ 11″	153— 3	158— 5	163— 8	168—10
6′ 0″	158— 3	163— 6	169—10	174—12
6′ 1″	163— 3	168— 6	175—12	180—14

NORMAL WEIGHT—34 TO 54 YEARS

AGES	35—39	40—44	45—49	50—54
HEIGHT	M—F	M—F	M—F	M—F
4′ 10″	125— 5	128— 4	130— 3	131— 2
4′ 11″	127— 5	130— 4	132— 3	133— 2
5′ 0″	129— 5	132— 4	134— 3	135— 2
5′ 1″	131— 5	134— 4	136— 3	137— 2
5′ 2″	133— 4	136— 3	138— 2	139— 1
5′ 3″	136— 4	139— 3	141— 2	142— 1
5′ 4″	140— 4	142— 3	144— 2	145— 1
5′ 5″	144— 4	146— 3	148— 2	149— 1
5′ 6″	148— 4	150— 3	152— 1	153— 1
5′ 7″	152— 4	154— 3	156— 1	157— 0
5′ 8″	157— 5	159— 4	161— 2	162— 0
5′ 9″	162— 6	164— 5	166— 3	167— 1
5′ 10″	167— 8	169— 7	171— 5	172— 2
5′ 11″	172—10	175— 9	177— 7	178— 4
6′ 0″	178—13	181—12	183—10	184— 7
6′ 1″	184—16	187—15	190—13	191—10

Female weight, subtract number of pounds designated.

I. NORMAL BODY WEIGHT IN POUNDS AND KILOGRAMS

The normal body weight in pounds is obtained from an age-height-weight actuarial table (Table I) in which the figures represent weights for males, female weights being obtained by subtracting the small figures opposite each weight.

The conversion table (Table II) converts this figure from pounds to kilograms by dividing by 2.2.

TABLE II

CONVERSION TABLE

BODY WEIGHT		BODY WEIGHT		BODY WEIGHT	
LB.	KILO.	LB.	KILO.	LB.	KILO.
15.4	7	83.6	38	151.8	69
17.6	8	85.8	39	154.0	70
19.8	9	88.0	40	156.2	71
22.0	10	90.2	41	158.4	72
24.2	11	92.4	42	160.6	73
26.4	12	94.6	43	162.8	74
28.6	13	96.8	44	165.0	75
30.8	14	99.0	45	167.2	76
33.0	15	101.2	46	169.4	77
35.2	16	103.4	47	171.6	78
37.4	17	105.6	48	173.8	79
39.6	18	107.8	49	176.0	80
41.8	19	110.0	50	178.2	81
44.0	20	112.2	51	180.4	82
46.2	21	114.4	52	182.6	83
48.4	22	116.6	53	184.8	84
50.6	23	118.8	54	187.0	85
52.8	24	121.0	55	189.2	86
55.0	25	123.2	56	191.4	87
57.2	26	125.4	57	193.6	88
59.4	27	127.6	58	195.8	89
61.6	28	129.8	59	198.0	90
63.8	29	132.0	60	200.2	91
66.0	30	134.2	61	202.4	92
68.2	31	136.4	62	204.6	93
70.4	32	138.6	63	206.8	94
72.6	33	140.8	64	209.0	95
74.8	34	143.0	65	211.2	96
77.0	35	145.2	66	213.4	97
79.2	36	147.4	67	215.6	98
81.4	37	149.6	68	217.8	99
				220.0	100

2.2 lbs. = 1.0 Kilo.

II. CALORIC ALLOWANCE PER KILOGRAM

For an average male diabetic patient, 30 calories per kilogram normal body weight are allowed; for an average female diabetic patient, 25 calories per kilogram normal body weight. These figures are used for the average type of diabetic individual whose work is light in character, as is true of the business and professional worker and the housewife. Their life is spent mainly indoors, their greatest exertion that of walking about. More calories are unnecessary for such a man, and women usually require less calories than men.

Based on physical activity, the following allowances are used per kilogram normal body weight.

For a bed patient, 25 calories
For light work, 30 calories
For medium work, 35 calories
For heavy work, 40 calories

III. TOTAL CALORIC ALLOWANCE

This is obtained very simply by multiplying I by II; i.e., kilograms normal body weight multiplied by caloric allowance per kilo yields total caloric allowance per day.

IV. THE DIET FORMULA

This is the simple method used to convert the total caloric allowance into carbohydrate, protein, and fat.

Protein.—For a man, one gram per kilo normal weight is allowed. For a woman, either three-quarters or two-thirds of a gram per kilo is sufficient. More than a gram of protein per kilo is unnecessary and wasteful. Less than two-thirds gram per kilo may be detrimental in not supplying enough protein to replace normal breakdown of muscle structure. It is well known that we in the United States consume a large per capita proportion of meat, much more than is physiologic. For the diabetic person, re-education in the matter of meat eating is a necessity. And in these days of meat rationing, a little re-education in how much meat we really need per day would be serving two purposes, nutritional and patriotic.

Fat.—In order to keep the diet as close as possible to its 20 per cent of caloric content in fat, an arbitrary limit on the fat in the diet must be set. The average American likes his fat and hence eats too much of it, and it also is a rationed item today. Therefore, the fat in our diabetic diet has been fixed at a maximum of 90 grams per day. This figure allows enough butter, cream, and milk for the average individual, and more is hardly ever necessary. Figures as low as 70 grams may be used without resorting to artificial fatlike substances such as mineral oil for frying and salads. But diets using as little as 40 or 50 grams are unsatisfactory to the patient and are usually abandoned by him soon after leaving the doctor's immediate supervision in the hospital.

Carbohydrate.—Having determined the protein and fat in the diet, the remainder of the calories are simply prescribed as carbohydrate.

Such a calculation takes about a minute or two for any patient. The procedure is best illustrated by an example, taking the steps in their proper order.

Assuming we are dealing with a patient who is 42 years old, 67 inches tall, and is a salesman by occupation:

1. Normal body weight should be 154 pounds or 70 kilograms.
2. Caloric allowance is 30 calories per kilo.
3. Total daily caloric allowance is $70 \times 30 = 2100$ calories.
4. Protein: 1 gram per kilo = 70 grams.
   Fat: = 90 grams
   Protein calories = $70 \times 4 = 280$ calories
   Fat calories = $90 \times 9 = 810$ calories
   280 calories (protein) + 810 calories (fat) = 1090 calories
   Total daily caloric allowance = 2100 calories
   Calories supplied by protein and fat = 1090 calories
   Calories to be supplied as carbohydrate = 1010 calories
   Carbohydrate (grams) = 1010 divided by 4 = 250 grams.

Diet: carbohydrate, 250; protein, 70; fat, 90.

INSULIN

The patient is given his maintenance diet, as outlined, for a period of four or five days, without insulin if possible. This is done in an attempt to evaluate the degree of severity of the patient's diabetes. In this hospital, we have arbitrarily set up three grades of severity of diabetes to match the Army's classification of three degrees of severity for most of the chronic diseases. The means used to control the patient's diabetes serves as the index of severity, as follows:

Mild, a diabetic individual requiring no insulin.
Moderate, a diabetic individual requiring up to 40 units insulin daily.
Severe, a diabetic individual requiring more than 40 units insulin daily.

In our experience, about one-third of the cases fall into the mild group in civilian practice. Our Army experience indicates that about half are mild cases, and most of the other half fall into the moderate group. This difference between diabetics in civilian and Army practice may be due to the fact that diabetics in the Army are discovered very early and that these cases receive prompt and adequate treatment in a hospital. It may be that this tends to halt or delay the natural tendency of the diabetes to progress and become more severe. Many such cases who have received insulin at some other Army hospital have been found to require less and in many cases no insulin after treatment at our hospital. A more detailed study of these cases is being prepared for publication at some future date.

Early in his hospitalization, the patient is taught to test his urine for sugar four times daily, once before each meal and on retiring, and to keep a record of these tests. In addition, he collects and measures his twenty-four hour urine output daily, and a quantitative analysis of the sugar present is done. The volume of urine in cubic centimeters multiplied by the per cent of sugar present yields the amount of sugar in grams that the patient is excreting daily.

Insulin dosage is based on this figure of grams of glucose excreted daily. All patients requiring insulin are started on protamine zinc only, allowing as a preliminary amount one unit of insulin for every 2 grams of glucose excreted. However, it is good policy not to prescribe less than 10 units of insulin daily. In other words, a patient excreting less than 20 grams of glucose per day usually can be made sugar-free by readjusting the carbohydrate among his three meals. This is where the four daily urine tests are of inestimable value. Ordinarily the patient receives three meals that are equal in carbohydrate, protein, and fat content. Most patients adjust themselves to this regime without difficulty. However, for the patient who is showing considerable sugar persistently after any one of the three meals, a slight adjustment in his meals may be made. This consists in shifting ten to twenty grams of carbohydrate from that meal to another, to a time of day when he is sugar-free. An adjustment of this sort has saved many a patient either from taking any insulin whatsoever or from an additional dose of another kind of insulin.

In our experience, few patients who require insulin are ever completely controlled on protamine zinc alone. In many cases, an additional dose of regular or crystalline insulin is necessary. We have only two requirements to propose when additional insulin is to be given. One is that this additional insulin dose shall never be more than one-third of the total insulin dose per

day and the other is that the additional insulin be given at breakfast time with the protamine zinc insulin rather than at some other time of day. We have always given the two kinds of insulin in the same syringe. In our experience, the effect is the same as when the two kinds of insulin are given separately. The only precaution to be observed is that of withdrawing the clear insulin first, followed by the protamine zinc insulin, so as to avoid injecting protamine into the other insulin bottle.

For the patient taking protamine zinc insulin, one other precaution is routine in this hospital. All such patients receive a small meal at bedtime, containing milk, to avoid depression of the blood sugar during the night to the reaction level.

A few words about blood sugars: They are taken three times weekly, before breakfast, in all diabetic patients. This is an extremely worth-while precaution in a patient taking protamine zinc insulin. Such a case may suffer insulin reactions, if blood sugars are not done. Treating diabetes without doing blood sugars is like making a suit of clothes without a pattern or a model. Each case must be individualized and all factors must be analyzed to achieve the maximum results.

Herein are graphically presented three cases of early diabetes seen at our hospital recently. They illustrate what the proper diet does, with and without insulin.

CASE 1 (Fig. 1).—This was a case of a 21-year-old soldier whose diabetes was discovered while in India, about one month after symptoms began. Note that his glucose tolerance had a peak of over 400 mg. blood sugar. He was placed on a high-fat diet: carbohydrate, 60; protein, 60; fat, 150; calories, 1,730. On this diet he was hungry and continued to spill sugar. Before being transferred to this hospital, his diet was raised to carbohydrate, 70; protein, 70; fat, 165; calories, 2,045. A second glucose tolerance test was better but still diabetic. He was still hungry. During this period of hospitalization he was sugar-free or showed only a trace of sugar for 26 out of his total of 40 days in the hospital.

He was transferred to this hospital on Feb. 14, 1943, and placed on a diet of carbohydrate, 230; protein, 70; fat, 90; calories, 2,010. This was about the same in total calories as his last diet in India. He was never hungry on this diet. A repeat glucose tolerance test showed more improvement. He was sugar-free or showed only a trace for 27 out of 28 days in the hospital.

Thus, despite a complete reversal of his dietary figures, representing an increase of 160 grams of carbohydrate over his previous diet, his diabetes was controlled better than before. And this increase of carbohydrate did not make necessary the addition of insulin. Finally, we had a satisfied patient.

CASE 2 (Fig. 2).—This was a case of a 36-year-old colored soldier whose diabetes was discovered about 6 weeks after symptoms began at a station hospital in a southern camp. He was placed on a diet of carbohydrate, 124; protein, 77; fat, 132; calories, 1,992. In addition, he was given regular insulin, four times daily, for a total daily dose ranging between 75 and 100 units. He was sugar-free or had only a trace of sugar for only 3 out of 23 days' hospitalization.

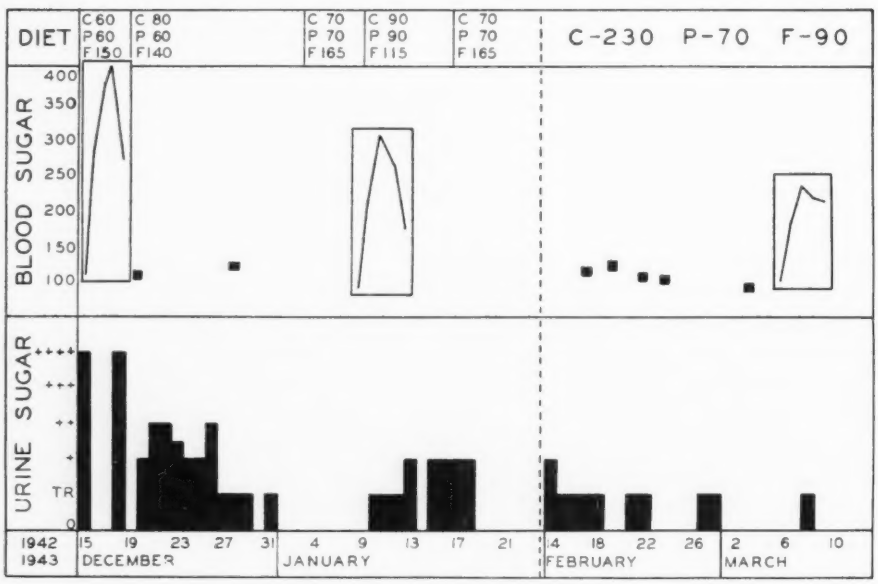
DIET
C 60 P 60 F 150
C 80 P 60 F 140
C 70 P 70 F 165
C 90 P 90 F 115
C 70 P 70 F 165
C-230 P-70 F-90
BLOOD SUGAR
400
350
300
250
200
150
100
URINE SUGAR
++++
+++
++
+
TR
0
1942
1943
15 19 23 27 31
DECEMBER
4 9 13 17 21
JANUARY
4 18 22 26
FEBRUARY
2 6 10
MARCH

Fig. 1.

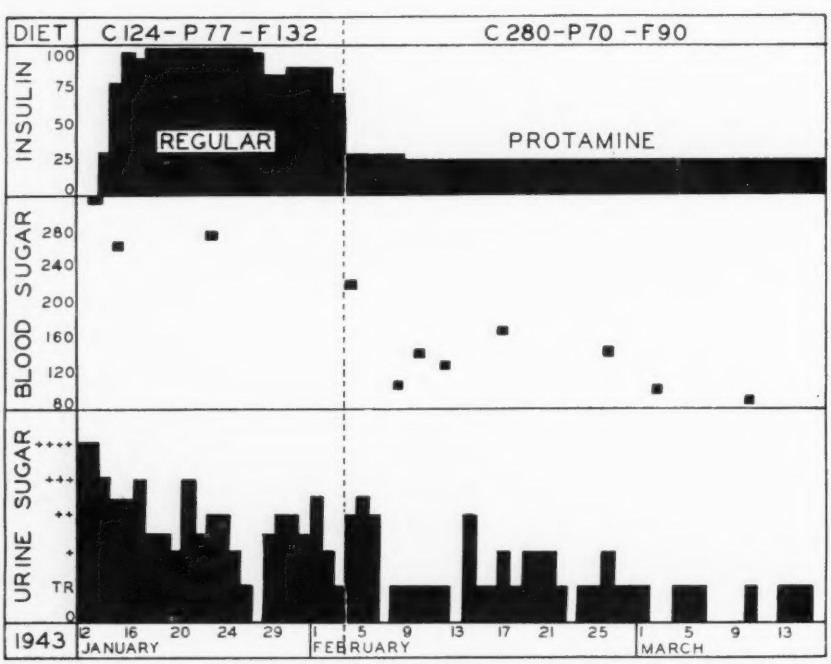
DIET
C 124 - P 77 - F 132
C 280 - P 70 - F 90
INSULIN
100
75
50
25
0
REGULAR
PROTAMINE
BLOOD SUGAR
280
240
200
160
120
80
URINE SUGAR
++++
+++
++
+
TR
0
1943
2 16 20 24 29
JANUARY
1 5 9 13 17 21 25
FEBRUARY
1 5 9 13
MARCH

Fig. 2.

He was transferred to this hospital on Feb. 4, 1943, and placed on a diet of carbohydrate, 280; protein, 70; fat, 90; calories, 2,210. Since he was admitted on a dose of 75 units of insulin daily, it was felt wiser to give him 30 units of protamine zinc insulin, once daily, rather than omit insulin entirely. This dosage was reduced in five days to 25 units. His blood sugar promptly became normal. He was sugar-free or showed only a trace for 32 out of 41 days' hospitalization. An additional dose of regular insulin would have kept him completely sugar-free, but owing to the difficulties involved in teaching him insulin administration, it was felt wiser not to attempt to burden him with this extra task.

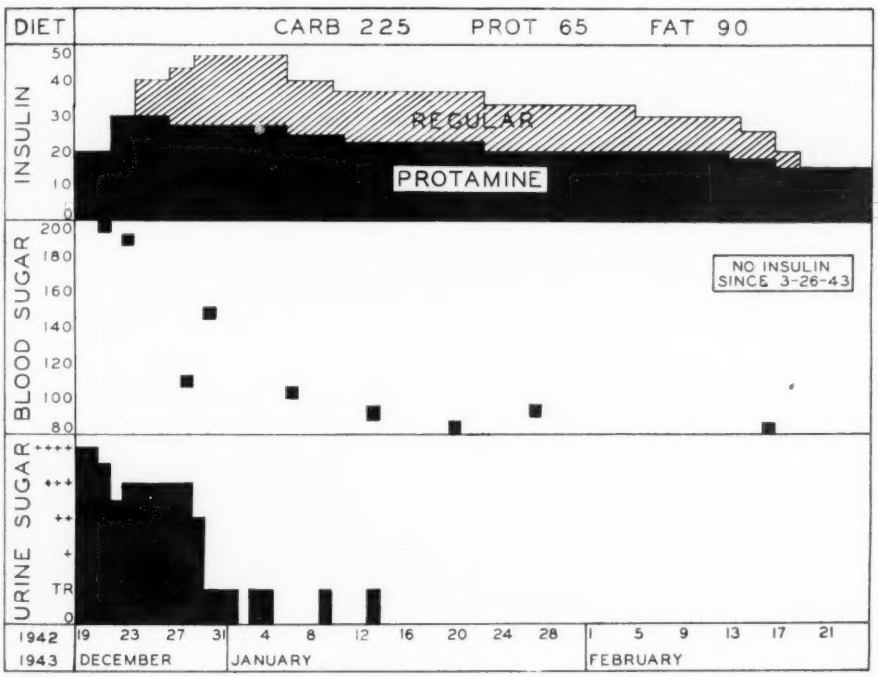

Fig. 3.

Case 3 (Fig. 3).—This was a case of a 32-year-old soldier whose diabetes was discovered about 3 weeks after symptoms began, at this hospital. He was placed on a diet of carbohydrate, 225; protein, 65; fat, 90; calories, 1,970. At first he was given protamine zinc insulin alone, and after five days, regular insulin was added, to control after-breakfast glycosuria. After ten days of hospitalization, he was sugar-free with a normal blood sugar on 48 units of insulin daily. His insulin was then gradually reduced, and he was discharged on 20 units of protamine zinc insulin daily, still sugar-free. Subsequently he was followed for a time and his insulin progressively reduced, until he was on diet alone and sugar-free. His insulin was discontinued completely three months after his diabetes was first treated. This is not an uncommon occurrence in fresh cases of diabetes that are treated early with insulin. Whether it bespeaks a "cure" of such diabetes is still a problem for the future to decide.

However, it does indicate that those patients that require insulin should be given enough insulin to bring them quickly under control. Rather than increase the insulin dosage gradually to a maximum, we prefer to give the maximum insulin dose at the beginning of treatment and decrease it later, as necessary, thus perhaps discontinuing it entirely in the course of a few months.

SUMMARY

1. The diabetic diet should be a physiologic diet and based on known values of the normal diet.

2. The diabetic diet should be prescribed for the individual patient, according to his needs.

3. The diabetic diet is based on normal body weight, obtained from age-height-weight tables, and on the degree of physical activity of the patient.

4. The total caloric allowance is quickly and simply calculated for each individual patient.

5. By a simple method, the total caloric allowance per day is divided into carbohydrate, protein, and fat, in accord with physiologic values for a normal diet.

6. The principles and procedure for prescribing insulin are simplified.

7. Finally, three cases of early diabetes treated by these principles are graphically presented, illustrating the simplification of the treatment of diabetes.

CIRCA 42, A NEW ITCH REMEDY*

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THIS is a report of a new itch remedy, called Circa 42, designed to relieve the itching of skin when applied externally. It has been developed in response to an acute wartime need of a means of preventing or alleviating skin irritations caused by the attacks of insects such as chiggers, mosquitoes, etc., and of lessening thereby the danger of infection from scratching. At the authors' request, a number of persons suffering from chigger bites or itches that arose in other ways have tested Circa 42 on themselves. Written records indicate that Circa 42 has considerable promise as an efficacious itch remedy and should be brought to the attention of those interested in the treatment of pruritic skin.

The composition of Circa 42 is

n-butyl-p-aminobenzoate†	100 Gm.
benzyl alcohol	170 c.c.
anhydrous lanolin (melted)	20 c.c.
cornstarch	640 Gm.
sodium lauryl sulfonate	64 Gm.

plus additional benzyl alcohol as indicated below. The n-butyl-p-aminobenzoate is dissolved in the benzyl alcohol, which is first warmed, making an approximately saturated solution. The melted lanolin is added, and the mixture is kept warm and stirred until as much of the lanolin as will dissolve is in solution. While the liquid is warm, it is added slowly, a little at a time, to a thorough mixture of the cornstarch and the sodium lauryl sulfonate, and the whole is carefully kneaded to distribute the liquid evenly throughout the powder. An additional amount of benzyl alcohol, about a tenth of that already used, is added as before to give the material a desired consistency. The final preparation should be a doughy, nongreasy, cakelike material that can be packed in ointment jars or other suitable containers, although it is better not to use containers made of metal.

When the remedy is to be applied, the skin may be moistened slightly with water, depending on the physical consistency of the Circa 42. This material is rubbed onto the skin until it forms a moderately thick layer over the affected region. No attempt should be made to rub it vigorously *into* the skin. It will gradually dry to a powder, which should be left on the skin undisturbed. Effectiveness of treatment is lost when the material is removed from the skin by sweating, bathing, friction of clothes, etc.

The benzyl alcohol penetrates the skin quickly and produces a local anesthesia of short duration. The n-butyl-p-aminobenzoate penetrates more slowly, but has a more prolonged local anesthetic effect. When the alcohol of Circa 42

*From the U. S. Department of Agriculture, Bureau of Entomology and Plant Quarantine, Agricultural Research Administration.

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†Sold under the name "Butesin."

enters the treated skin, it takes dissolved benzoate in with it. The combined anesthetics produce a quick and prolonged local anesthesia. The lanolin helps to soften the skin and favors penetration, the sodium lauryl sulfonate facilitates spreading, and the cornstarch acts as an inert carrier base.

Records were obtained from 32 persons suffering from chigger bites and only 2 failed to obtain any relief. A total of 83 treatments were made. Of 76 records indicating quickness of relief, 61 showed that relief was obtained in less than 15 minutes, 13 in 15 to 30 minutes, and 2 in more than 30 minutes. Of 69 indicating duration of relief, 14 showed that relief persisted for less than 5 hours, 15 for 5 to 8 hours, and 40 for more than 8 hours; of the last, 13 showed that relief lasted from 8 to 48 hours, and 27, that the relief was permanent. In 18 instances permanent relief was obtained after only 1 application of the material; in 6 instances, after 2 applications; and in 2 instances, after more than 2 applications. Six of the 83 records did not indicate speed and duration, but all showed that some relief was obtained; 8 were interrupted by loss of the material from the skin because of bathing, sweating, and friction from clothes, but not before some relief from the itching was obtained.

The material was tested also on skin itching from other causes. Seven persons reported on 14 treatments made for relief of mosquito bites; 7 of these treatments were each followed by relief in less than 15 minutes, 6 in 15 to 30, and 1 in more than 30 minutes. Each of 9 treatments gave relief lasting less than 5 hours, and each of 4 gave permanent relief.

Permanent relief of discomfort from a yellow jacket sting was reported by one person, the relief occurring in less than 15 minutes. Another person treated "spider" bites. Each of 4 treatments gave relief in less than 15 minutes; 3 gave relief for 5 to 8 hours, and permanent relief from 1 bite was had after 2 treatments.

Five persons reported on use of the material to relieve itching from unrecognized causes. Of the 11 treatments, 9 gave relief in less than 15 minutes, 1 in over 30 minutes, and 1 gave no relief at all; 1 gave relief lasting less than 5 hours, and each of 8 treatments gave relief for over 8 hours. In two instances the relief lasted indefinitely, in one case after 1 treatment, in the other, after 2.

Two persons reported on treatment of poison ivy itch with Circa 42. Each of 2 treatments gave relief in less than 15 minutes, and each of 2 others in 15 to 30 minutes. Relief lasted less than 5 hours in 2 instances, 5 to 8 hours in 1, and indefinitely (after a single treatment) in 1 instance.

Similar results were indicated verbally by a number of other persons who used Circa 42 to relieve itching from chigger bites, mosquito bites, poison ivy, and unknown causes.

Equally promising results have been obtained by personnel of the United States Army,* who have made preliminary tests wherein Circa 42 was used in the tropics to relieve itching caused by chigger bites and fungus infections.

Conclusions.—These tests indicate that Circa 42 shows considerable promise as a remedy for the relief of itching skin and is worthy of more extensive trials by those interested in the treatment of pruritus.

*Permission to refer to those results has kindly been granted by the Office of the Surgeon General, Washington, D. C.

CLINICAL USE OF ORAL THERMOMETERS*

Report of Study to Determine Time Required for Reliable Registration

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ALTHOUGH many scientific papers have been written on the subject of clinical thermometers, one question has remained unanswered: What is the basis for the time designations ("1 minute," "½ minute," "60 seconds," etc.) used on oral clinical thermometers? In an effort to gather information on this subject, a questionnaire was sent to 100 training schools for nurses throughout the country. This questionnaire asked: (1) "What is the minimum time interval between insertion and reading of clinical thermometers?" and (2) "Have the above time intervals been selected on the basis of the manufacturers' statements, your own clinical tests, common knowledge, or some other basis?" Of the 69 replies to the first question, 27 answered that the time of insertion of the thermometer in recording oral temperature was less than three minutes; 37 stated three minutes; and 5 recommended longer than three minutes. In reply to the second question, 13 stated that these time intervals were based partially or wholly on manufacturers' statements; 30 on clinical test; 26 on common knowledge; 16 on textbook statements, and 10 on experience. Several of the replies to the second question indicated that the basis of selection of a time interval had been obtained from more than one source. Since there seemed to be such wide variance among professional persons most familiar with the taking of oral temperatures, and since the time designations on the instruments have been interpreted by lay users to indicate the time necessary for registration of the actual temperature of the patient, it was decided to undertake a study to determine the time required for oral clinical thermometers to reach equilibrium† in the mouths of individuals under ordinary conditions of use.

Approximately 1,000 clinical thermometers were collected by the Food and Drug Administration throughout the United States in the Spring of 1940. Of these thermometers, about 800 were found to meet the requirements and tests specified for clinical thermometers in the Commercial Standard CS 1-32 of the United States Department of Commerce, Bureau of Standards. By random sampling of these 800 thermometers, 20 instruments were selected for testing.

Realizing at the outset that there might be wide variations in the time required for individual instruments to reach equilibrium, preliminary investigations were conducted to ascertain the effect of various media with different conductivity coefficients on the form of the "time-temperature curve" for oral

*From the Food and Drug Administration, Federal Security Agency, Washington, D. C. Received for publication, July 29, 1943.

†Equilibrium of an instrument was considered for our purposes to be that temperature reading within one-tenth of one degree of the registration of the instrument after five or six minutes of insertion in the media.

clinical thermometers. In these preliminary studies simultaneous observations of time and temperature readings on oral clinical thermometers were made under laboratory conditions in different media at various temperatures. Subsequently, the clinical experiment was undertaken. The details and results of both the laboratory and clinical experiments are given below:

WATER BATH EXPERIMENT

These 20 thermometers were placed, in pairs, in a water bath which was maintained at a constant temperature by means of a thermostat and a device to keep the water in circulation. A reading was taken of each instrument every thirty seconds for six minutes.* The bath was maintained at 98° F. until all the thermometers were read, then at 100° F., 102° F., and finally 104° F. Conditions were carefully controlled so that as few extraneous factors as possible would influence the results. Some of these factors were: depth of immersion of the thermometer in the water, angle of vision while reading the instrument, interval of testing during the heating cycle of the bath, and the human factor (all readings by the same person) in reading the instrument.

It was noted that the mercury rose rapidly, and although each instrument reached equilibrium within ten seconds, the water bath experiment gave certain information concerning the variation of the final readings among thermometers. It appeared that the thermometers were slightly more variable at lower temperatures since the standard deviation at 98° F. was 0.13° and decreased as the temperature was increased, until at 104° F. it was 0.09.° (The ratio of these variances is just significant statistically and indicates a possible trend.)

As the rise of mercury in the thermometers was too rapid in this medium to obtain a satisfactory "time-temperature curve," it was decided to repeat the experiment in another medium with a lower conductivity coefficient.

AIR-BATH EXPERIMENT

A practical medium with a lower conductivity coefficient than water is air. Testing was carried out in a thermostatically controlled air bath, with the temperature maintained at 106° F. The 20 thermometers were inserted in the air bath singly. The time required for the mercury column to rise from 94° F. to 95, 96, 97, 98° F., etc., was recorded. It was observed that all the thermometers reached equilibrium in ninety seconds. Nevertheless, the thermometers were retained in the air bath for six minutes to make certain that the instruments had reached equilibrium. Each instrument was rechecked after an initial test. Such factors as depth of insertion of the instrument in the air bath, angle of vision while reading the instrument, interval of testing during the heating cycle of the bath, and the human factor in reading the thermometer were controlled.

The readings of the temperature and time in seconds are plotted for a single typical thermometer in Fig. 1. It is noted that about one and one-half minutes were required for an oral clinical thermometer to reach equilibrium in an air bath. The repeated observations on the same thermometer closely dupli-

*All timing throughout the investigation was done with a stop watch calibrated in two-tenths of a second.

cate those of the initial readings. Considering the actual data and theoretical aspects, a smooth curve was fitted to the data. It is of the form

$$Y = T_f - (T_f - T_i)\ e^{-bx}$$

where T_i is the initial temperature reading of the thermometer (here 94° F.), T_f is the final temperature, and Y is the temperature at any given time (x).

In terms of logarithms, the equation means that a straight line with slope "b" could be fitted to plotting of the logarithms of the relative change in temperature against time.

All 20 thermometers were tested in the same manner, and it was found that this type of equation was the best fitting in each instance.

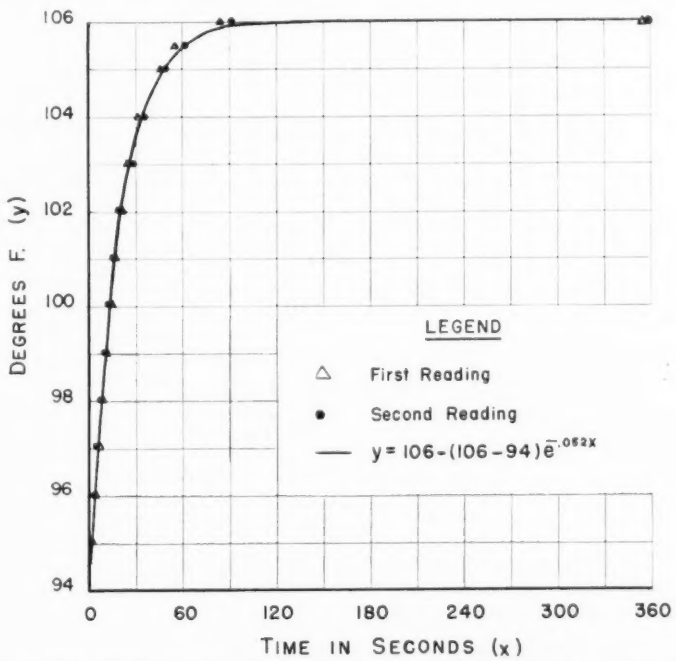

Fig. 1.—Curve fitted to thermometer readings in air bath constant at 106° F.

CLINICAL TESTING

From these determinations in the air bath, the 20 thermometers under consideration were arbitrarily classed as "slow" and "fast." It was noted that some of these instruments bore a time designation and that about half were of the "regular" bulb type and the remainder "stubby" bulb instruments. Consequently the 8 thermometers picked from the group of 20 for use in our clinical study were separated into 8 groups according to these three physical characteristics. This choice and classification were made primarily to insure that the various types of oral thermometers were equally represented in the clinical test.

Since it was realized that physiologic variations in individual subjects might influence the recording time, a preliminary clinical investigation in which a thermometer was inserted in a patient's mouth for periods of from one second to ten minutes showed that it is possible to repeat temperature readings closely even when the thermometer is inserted for less than one minute. However, these readings were not the patient's "actual" temperature as indicated

by the reading of the instrument after five or six minutes. The point to which the mercury was shaken down seemed to make little difference as long as the meniscus was below 95° F. From this experience, the following "time intervals" were chosen for use in the clinical testing of the 8 thermometers: (1) five seconds; (2) ten seconds; (3) twenty seconds; (4) thirty seconds; (5) forty-five seconds; (6) sixty seconds; (7) ninety seconds; (8) one hundred and twenty seconds; (9) one hundred and eighty seconds; (10) two hundred and forty seconds; (11) three hundred seconds; (12) three hundred and sixty seconds.

It was noted during these preliminary clinical studies that certain activities appeared to affect the registration of the temperature. Taking hot drinks and smoking produced transitory elevation, and the taking of cold drinks caused a temporary lowering of local mouth temperature, which required from fifteen to thirty minutes to return to "normal." Consequently, suitable precautions were taken to control the subject's activities directly before and during the regular periods of testing.

In the clinical study of a group of 16 healthy girls, 8 white and 8 Negro, and a group of 16 healthy boys, 8 white and 8 Negro, all between the ages of 14 and 20 years, were used. Since the subjects were domiciled in training schools, their activities and diets were comparable throughout the periods of testing. These 32 subjects were taught the proper method of using an oral clinical thermometer. The 8 selected thermometers were tested and rechecked on all the subjects in both groups. The thermometers were read, temperatures recorded, and the instruments shaken down by us to a point near their original readings (below 95° F.) after each of the specified time intervals. One hour and fifteen minutes were required to complete an initial and recheck "set of readings" for each subject with any one thermometer. Each subject was tested about the same time of day in order to reduce the possibility of encountering the diurnal variation of the person's temperature.

The summary of the clinical tests consisted of 493 "sets of readings,"* or 5,916 individual readings. (A "set of readings" is one subject's temperature record at all of the 12 time intervals as registered by any one instrument.)

The "time-temperature curve" for a single thermometer on a single subject was not as smooth as that for a single thermometer in the air bath. This is undoubtedly due to a slight physiologic variation in the subject and/or variations in the contact between the instrument and the surrounding tissue. However, an average "time-temperature curve" for several subjects on several thermometers is a smooth curve, although of slightly different type from that obtained when the thermometer is tested in an air bath.

Fig. 2 shows the average of the 493 readings plotted for each time interval, a smooth curve fitted to these averages, and, for comparison, a curve of the type applied to the data from the air bath experiment. The difference between the two curves can be seen readily. Although both curves seem almost identical for the first sixty seconds, the air bath curve continues in a horizontal line, while the curve for the averages of the clinical data continues to rise. This continued rise of the curve fitted to the clinical data may be due to a delayed rise of the mercury column in the thermometer caused by the slow return of

*Because of circumstances beyond our control, only 493 complete "sets of readings" were obtained.

the tissues to body temperature after a possible slight cooling of these tissues on initial contact with the thermometer.

Having determined the "time-temperature curve" of a clinical thermometer under clinical conditions and having demonstrated the difference between the "time-temperature curves" for laboratory and clinical conditions, we concluded that we could proceed to determine the length of time necessary for a thermometer to approach within one-tenth of one degree of the "final" temperature under clinical conditions. "Final" temperature, for our purpose, is the average of the five- and six-minute readings of each set for each individual. Within each set of readings for 1 thermometer and 1 subject, differences were taken between each reading and its own "final" temperature. As will readily be seen, by using these differences the variation between thermometers and the variation between individuals are eliminated. This permits the tabulation of the difference between the actual reading at any given time interval and the reading finally attained.

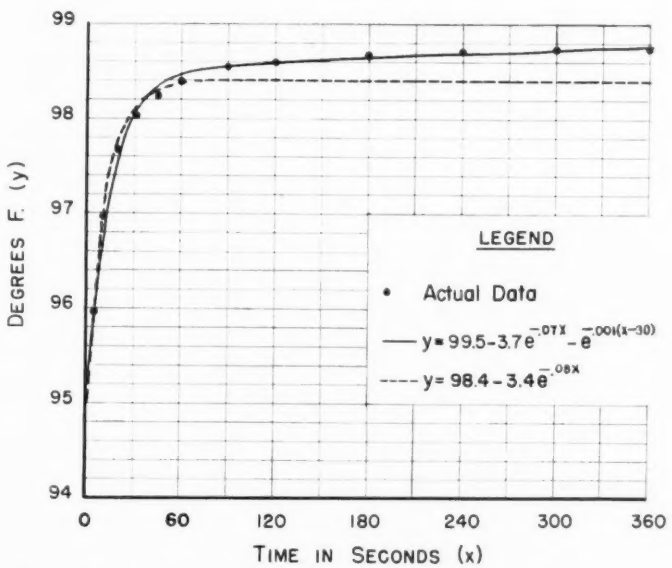

Fig. 2.—Curve fitted to averages of 493 "sets of clinical readings" as compared with a theoretical curve of the type derived from air bath data.
unbroken line, clinical reading.
broken line, theoretical curve derived from air bath data.

It can be seen from Table I that an oral clinical thermometer should remain in the mouth for three minutes (one hundred and eighty seconds), since the average of the 493 determinations required that amount of time to come within one-tenth of one degree of the final reading. In this study, in which 32 persons were used, the average three-minute temperature of the boys is 98.4° F. while that for the girls is 99° F.

A detailed statistical study of the differences from the "final" temperature by analysis of variance demonstrated that differences between sexes or between subjects were statistically significant in this clinical study. The differences between thermometers, however, were not significant even when they were separated into two groups; namely, those marked "1 minute" and those having no specified time designation.

The readings on the thermometers marked "1 minute" did not approach their own "final" readings significantly faster than the unmarked thermometers. At the end of three minutes, the thermometers with no time designations had an average difference of 0.093° ± 0.010° F. from the "final" temperature reading, while the corresponding figure for those with time designations was 0.077° ± 0.011° F. The type of bulb did not seem to cause any differences in the time required to reach the "final" reading. These figures for thermometers with no time designations on "regular" and "stubby" bulbs were, respectively, 0.105° ± 0.013° and 0.081° ± 0.016° F., while for thermometers with time designations, the averages for "regular" and "stubby" bulbs were, respectively, 0.065° ± 0.015° and 0.088° ± 0.016° F.

TABLE I

AVERAGE DIFFERENCES, STANDARD DEVIATION OF DIFFERENCES, AND GREATEST DIFFERENCE FROM "FINAL" TEMPERATURE FOR EACH TIME INTERVAL FOR THE 493 SETS OF READINGS

TIME INTERVAL SECONDS	DIFFERENCE FROM SUBJECT'S FINAL TEMPERATURE		
	AVERAGE DIFFERENCE, DEGREES F.	STANDARD DEVIATION DEGREES F.	GREATEST DIFFERENCE, DEGREES F.
5	2.792	1.146	6.8
10	1.780	0.899	4.8
20	1.083	0.648	3.8
30	0.731	0.485	3.6
45	0.511	0.398	3.2
60	0.367	0.332	2.6
90	0.225	0.251	1.5
120	0.151	0.251	1.6
180	0.083	0.169	0.9
240	0.044	0.159	0.8*

*There was one very unusual difference greater than 0.8. This figure was 1.7.

APPLICATION OF RESULTS

On June 12, 1941, the Food and Drug Administration issued a trade notice to manufacturers and distributors of clinical thermometers in reply to numerous requests concerning the type of information that should appear on clinical thermometers and in the circulars accompanying the instruments. In order to obtain uniformity of information in the material to accompany the instruments which would not meet with adverse criticism under the provisions of the Federal Food, Drug, and Cosmetic Act, a circular entitled "Directions for Use of Thermometer" was provided as a guide. The text of this circular included information on how to read, shake down, use, and care for a thermometer and interpret temperature readings as well as information regarding the accuracy of the instrument. Copies of this circular may be obtained, upon request, from the Food and Drug Administration, Federal Security Agency, Washington, D. C.

CONCLUSIONS

1. The character of the rise of the mercury column in an oral thermometer under clinical conditions is different from that under laboratory conditions.

2. In this study, thermometers marked with specific time designations "1 minute," "½ minute," and "60 seconds" were found to require about the same length of time for the instrument to reach equilibrium as thermometers with no time designations.

3. Three minutes should be the minimum time interval allotted for an oral thermometer to reach equilibrium under ordinary conditions of use.

ELLIPTOCYTOSIS, A REPORT OF TWO CASES*

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ELLIPTOCYTOSIS is a rare morphologic anomaly of human red blood cells. The condition was first described by Dresbach[1] in 1904 and apparently has no pathologic significance. Wyandt and her associates[2] in 1941 presented a total of 332 cases, composed of 86 persons studied by them and 246 individuals previously reported. In the following report, two additional individuals, an American Negro baby and his father with this anomaly, are added to the list. A detailed study of blood of the baby indicated a progressive increase in the degree of elliptocytosis during the first four months of life.

A comprehensive bibliographical survey of elliptocytosis has been given by Wyandt, et al. and need not be repeated here. No additional references have been found in the literature.

The pertinent findings of our cases are outlined below:

CASE 1.—The patient, a male aged two and one-half months, was admitted to the Children's Hospital of Pittsburgh on August 30, 1942, with a history of diarrhea, malnutrition, and a fever of two and one-half days' duration. He was weak and dehydrated. During his seven weeks' stay in the hospital he gained weight slowly and, except for the period between October 10 and October 17, 1942, when he developed an upper respiratory infection and a bilateral otitis media, he maintained a subnormal temperature. Bacteriologic findings and urinalysis were irrelevant. At the time of his discharge on October 23, 1942, he appeared much improved. During the first routine blood examination a considerable number of oval red cells were discernible in the counting chamber and a tentative diagnosis of elliptocytosis was made. Blood examination during his period of hospitalization, together with two blood counts at a later date, is summarized in Table I. The leucocytosis occurring during the early hospitalization was probably related to otitis media. The cause of the lymphocytosis on the last examination was not ascertained.

TABLE I

DATE	HB. IN GRAMS	R B C IN MILLIONS	W B C IN THOUSANDS	NEUTROPHILES %	LYMPHOCYTES %	MONOCYTES %	MYELOCYTES %	METAMYELOCYTES %	EOSINOPHILES %
8/31/42	10.0	3.4	14.2	41	40	1	6	10	2
9/11/42	8.8	3.5	12.9	49	48	2	1		
9/30/42	9.2	3.75	11.7	69	25	1	1		4
10/14/42	7.5	3.1							
10/23/42	8.2	2.95	6.8						
12/ 1/42	8.1	2.9	9.1	54	40	1		4	1
4/ 1/43	10.0	3.61	14.1	27	69		1	2	1

On December 1, 1942, the patient, apparently well, was readmitted for further blood study. Blood platelets averaged 486,000 per c. mm. The reticulocytes showed a value of 1.8 per cent, and none of these appeared oval. Fragility tests gave a range of hemolysis between 0.50 per cent and 0.30 per cent sodium chloride. Increased oxygen and carbon dioxide tension and washing with lecithin produced no appreciable change in the appearance of the

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erythrocytes. Enumerated erythrocytes in stained smears were divided into three groups on the basis of the following measurements: namely, round cells (6 to 8.5 μ), oval cells (9 $\times$ 6 μ to 6.5 $\times$ 5 μ), and sausage-shaped cells (11 $\times$ 3.2 μ to 7 $\times$ 5 μ). Comparison of the percentile distribution of the cells in the three groups is shown in Table II.

TABLE II

DATE	HB. IN GRAMS	R B C IN MILLIONS	NO. CELLS COUNTED	% ROUND	% OVAL	% SAUSAGE
9/11/42	8.8	3.5	400	75	25	
9/30/42	9.2	3.75	400	67.5	32	0.5
10/14/42	7.5	3.1	400	76	22.5	1.5
10/23/42	8.2	3.75	400	33	59	8
12/ 1/42	8.1	2.9	2000	36	51.9	12.1
4/ 1/43			2000	34.6	52.6	12.8

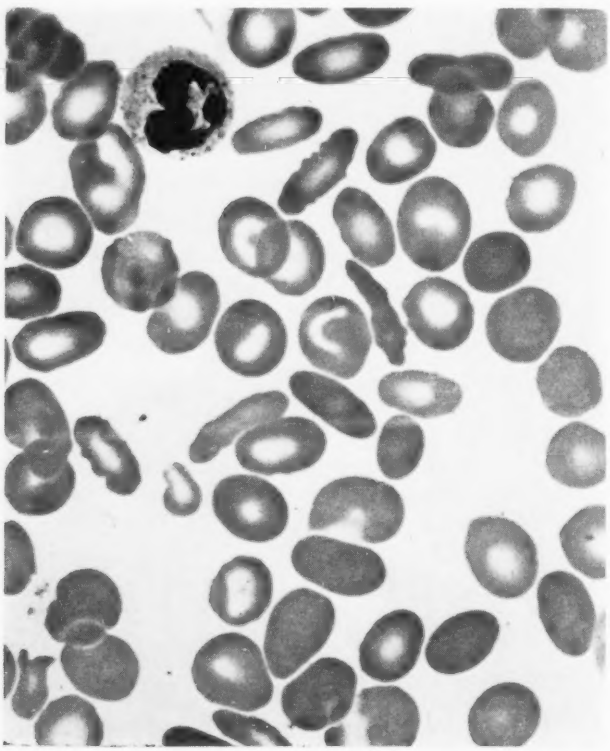

Fig. 1.—Photomicrograph of blood smear showing elliptical cells. (Wright and dilute Giemsa stain.) ×1200.

It is concluded that stability in the development of elliptocytosis was reached about the fourth month, because a progressive increase in the percentage of sausage-shaped cells was observed up to but not beyond this time (Fig. 1). After the fourth month, constant percentages of oval and sausage forms were obtained. The interruption in the progressive increase in degree of elliptocytosis shown on October 14, 1942, is probably due to infection. Sternal marrow showed all nucleated red cells to be round.

CASE 2.—The father of this patient, aged 25 years, was the only member of this family available for study who showed elliptocytosis. One complete blood count established the diagnosis, but no opportunity was presented to study his blood in detail. His stained smears showed no sausage-shaped forms, but a fair percentage of oval forms. Complete blood

counts made on the mother, a paternal uncle and his baby, and the paternal grandmother showed nothing of interest.

DISCUSSION

The most interesting feature was the progressive increase in the degree of elliptocytosis up to the fourth month, when the condition apparently became stabilized. Wyandt et al.[2] and Hunter[3] reported that the anomaly was present at birth with an increasing degree of elliptocytosis as growth of the child proceeded. Neither investigator stated when the degree of development was complete. Hunter was unable to follow the increased abnormality of the shape of cells beyond the third month because of the sudden death of the child.

A possible explanation for the decreased percentage of elliptical cells during infection, as shown by the count on October 14, 1942, may be the fact noted in transfusion experiments by Vischer[4] that these abnormal cells have a shorter life span of twelve to thirteen days, as compared with the approximate thirty days' life span of the normal red cells, and hence may be more readily removed from the circulation. This more rapid destruction would result in a greater number of young normal erythrocytes in the peripheral circulation and thus a relatively lower percentage of oval forms.

The nucleated red blood cells in the sternal marrow and reticulocytes in the circulating blood did not exhibit elliptical contour. This observation agrees with the findings of Cheney,[5] Leitner,[6] and Terry et al.[7] in contrast to those of Florman and Wintrobe[8] who noted 0.2 per cent elliptical reticulocytes in bone marrow. Altering environmental conditions, as with increased O_2 and CO_2, produced no alteration in the elliptocytes.

SUMMARY

Two cases of elliptocytosis are reported. In one of these, a progressive increase in the degree of elliptocytosis up to the fourth month was noted.

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CLINICAL CHEMISTRY

REACTION FOLLOWING INGESTION OF SODIUM BENZOATE IN A PATIENT WITH SEVERE LIVER DAMAGE

Severe Substernal Pain, Shock, Increase in Icterus, Granulocytopenia and Eosinophilia in a Colored Male Following Hippuric Acid Excretion Test

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THERE recently occurred in this hospital a reaction, following the ingestion of sodium benzoate for hippuric acid excretion test, in a patient with severe liver damage. This was of sufficient gravity to endanger his life. Three other patients with equally severe liver damage were given sodium benzoate simultaneously, from the same bottle, without untoward reaction. To test for impurities, six grams of the drug were given to a guinea pig but failed to cause any ill effect. In view of these findings and the generally accepted belief that sodium benzoate does not cause untoward systemic reactions, it was, at first, thought that the reaction was not due to the drug, so the test was repeated. This time there was an even more marked reaction, with severe substernal pain and shock, followed by granulocytopenia and an increase in the icteric index. The lack of case reports of severe reactions from sodium benzoate prompts the presentation of this case.

CASE REPORT

B. S. was a 24-year-old colored male admitted June 1, 1942, because of anorexia, postprandial epigastric distress, weakness, and dark urine, of four days' duration. His past health had been good and there had been no previous episodes of jaundice. He did not use alcohol or tobacco in any form. The admission examination revealed a well-developed and well-nourished colored male who appeared somewhat ill. There was moderate jaundice of the sclerae and mucous membranes. The heart and lungs were normal to percussion and auscultation, and the spleen and liver were normal to percussion and palpation. The stools were light, but contained some bile; the urine was quite dark and contained bile. The hemoglobin and red blood cell count were normal; white blood cell count, 5,850; differential: polymorphonuclear 60 per cent, eosinophiles 3 per cent, lymphocytes 32 per cent, monocytes 5 per cent; Kahn negative; icteric index, 57. The course of the patient's illness was gradually downhill for the first two weeks in spite of choline chloride, vitamin B complex, and diet rich in protein and carbohydrate, but after that he rapidly grew worse. On July 25, the patient was started on 6.4 mg. of vitamin K intramuscularly daily, because the prothrombin was 42 per cent of normal. By June 29, the liver dullness had diminished to 2 centimeters; the icteric index had mounted to 361; and a hemorrhagic tendency had de-

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veloped. The following morning, the patient was stuporous, and by afternoon was comatose. Marked bleeding now occurred after cleaning his teeth or after any minimal trauma to the mouth.

By the use of transfusions from donors who had received vitamin K, whole blood transfusions, plasma, vitamin K, and other measures, the bleeding was controlled and the patient improved. The prothrombin increased to near normal, the icteric index gradually diminished to 220 by July 15, and the patient appeared improved. On July 21, it was decided to attempt a hippuric acid excretion test in an effort to determine the functional capacity of the liver. The routine test, as devised by Quick,[2] was used, the patient being given 6 Gm. of sodium benzoate in 30 c.c. of water, by mouth. The test was uneventful until four hours after the administration of the sodium benzoate, when the patient suddenly complained of severe pain across his upper chest, "as if somebody was stabbing me with a knife," extreme dyspnea, and orthopnea. His blood pressure earlier in the morning was 106/70; but now it was 150/120 and the pulse was rapid and thready. The patient rapidly went into shock, with the pulse becoming imperceptible, and lapsed into a semicoma; then by the use of plasma, oxygen, and stimulants, he recovered from the shock and felt better within a few hours. Electrocardiogram, x-ray of the chest, physical examination, urine, chemical studies of the blood, and other examinations were all essentially as before the test, except that the icteric index had gone up to 275. The following day, on July 22, the patient's condition was further improved, and repeated studies failed to reveal a cause of the episode.

A rather rapid survey of the literature revealed no report of untoward systemic reactions from sodium benzoate, and it was thought that the episode was incidental and not due to the drug. The first test was not completed, and as the condition of the patient was satisfactory, the test was repeated on July 23, the patient again being given 6 Gm. of sodium benzoate in 30 c.c. of water, by mouth. At approximately the same time, four hours after ingesting the chemical, the patient again complained of severe chest pain, this time across the lower chest. Extreme dyspnea and orthopnea occurred a few minutes later, with the blood pressure rising to 155/126. The patient again rapidly went into shock, requiring intravenous glucose, plasma, oxygen, and stimulants for relief. During the shock his condition resembled that of a patient with a rather large pulmonary embolus. He could not be aroused for about twenty minutes and was stuporous and confused for four hours. Again the patient made a recovery from the shock, but this time only partially regained his former condition, feeling much weaker, appearing rather ill, and lacking his usual appetite and good spirits. Again, a thorough study was essentially as before the reaction, except for the icteric index, which had increased from 220 on July 15 to 275 on July 22, and to 285 on July 24. This was interpreted as an indication of increased damage to the liver, as there was no evidence of hemolysis of a large number of red blood cells. After the second episode, it was felt that both reactions were due to the sodium benzoate. This was further substantiated by the fact that the patient had received only vitamin K, vitamin B complex, choline chloride, transfusions, and plasma during his hospital stay, and continued to receive these during his recovery from each episode. The two reactions were essentially similar and occurred at almost exactly the same time after ingesting the drug.

After the first reaction, the white count was 4,450, with 40 per cent polymorphonuclears, 8 per cent eosinophiles, 7 per cent basophiles, and 46 per cent lymphocytes.

On July 26 he developed a cough, fever, sore throat, and malaise. Examination revealed mild redness of the throat and enlarged cervical glands. Otherwise, nothing was made out on physical examination aside from jaundice, small liver, and emaciation. The white blood cell count was 1,600, with 4 per cent eosinophiles, 1 per cent basophiles, 61 per cent lymphocytes, and 34 per cent monocytes. Repeated examinations of several smears showed no neutrophilic granulocytes. Tables I and II tabulate the white and differential blood counts, hemoglobin, red blood cell counts, liver, and transfusions during this period. One hundred units of liver extract and a 250 c.c. transfusion of whole citrated blood were given. On the following day, July 27, there were 2,400 white cells, again without any neutrophiles, the differential showing 5 per cent eosinophiles, 2 per cent basophiles, 58 per cent lymphocytes, and 35 per cent monocytes. Several checkup counts, including the various vital

TABLE I

BLOOD STUDIES

	DATE	HGB.	R.B.C.	W.B.C.	DIFFERENTIAL								
					MYELOB.	MYELOS.	JUV.	STABS.	SEGS.	EOS.	BAS.	LYMPHS.	MONOS.
1	6/ 2/42	92%		5,580	0	0	0	0	60	3	0	32	5
2	7/23/42	85%		4,450	0	0	0	0	40	8	7	45	0
3	7/26/42*			1,600	0	0	0	0	0	4	1	61	34
4	7/27/42*			2,400	0	0	0	0	0	5	2	58	35
5	7/28/42 A.M.	80%	4.08	2,650	0	0	0	0	0	4	1	60	35
6	(A.T.) P.M.	100%	4.58	3,200	0	0	0	0	2	9	0	39	50
7	7/29/42 A.M.	104%	5.50	4,700	0	0	2	2	1	5	2	74	15
8	P.M.			5,000	0	0	0	0	0	6	0	51	43
9	7/30/42 P.M.*	95%	4.14	5,000	6	30	0	0	9	7	2	31	14
10	7/31/42 A.M.	95%	4.72	7,050	1	30	4	4	13	8	2	24	15
11	P.M.	100%	4.71	7,350	1	12	1	14	11	7	4	41	9
12	8/ 1/42 A.M.	100%	5.33	7,500	0	10	3	6	30	17	2	28	4
13	(A.T.) P.M.		5.32	8,300	0	2	0	12	30	9	1	33	13
14	8/ 2/42	95%	5.20	8,150	0	2	5	11	23	6	0	40	13
15	8/ 3/42 A.M.	95%	5.50	6,500	0	0	3	8	38	6	1	31	13
16	P.M.			6,150	0	2	4	7	35	5	1	34	12
17	8/ 4/42 A.M.			8,350	0	0	0	0	62	1	2	35	0
18	P.M.			11,100	0	2	3	8	37	6	2	29	13
19	8/ 5/42	85%		14,700	0	4	6	11	49	2	1	24	3
20	8/ 6/42	90%	5.15	10,200	0	3	1	2	54	5	2	28	5
21	8/10/42 A.M.			20,350	0	0	0	0	91	0	1	9	0
22	P.M.	95%		36,600	0	0	2	27	66	0	0	5	0
23	8/11/42			18,950	0	0	0	25	70	0	0	5	0
24	8/12/42			14,100									
25	8/13/42			10,050	0	0	0	10	62	11	2	9	6
26	8/14/42			9,950	0	0	0	7	61	5	2	18	7
27	8/15/42			13,350	0	1	1	1	62	8	2	22	3

*Mean average of 9 complete counts.
A.T.—After Transfusion.

stains, continued to show complete absence of the neutrophilic series. The patient continued to receive whole blood transfusions, 200 units of intramuscular liver daily, and large amounts of all the vitamins.

On July 28, the white count was 2650 and was still without any of the neutrophilic series. The patient was much worse, the cough and fever had increased in severity, and the throat was now quite red and swollen, but without ulcers. The cough was productive of a greenish-yellow, foul sputum, with innumerable Vincent's organisms, but with no pus cells.

By July 29, the patient had not improved, and a sternal biopsy by the punch method was made. This revealed an arrested maturation of the myeloid elements of the bone marrow, except eosinophiles and basophiles. Peculiarly enough, these two constituents were found in normal or increased numbers, mostly in mature form. The amount of intramuscular liver injected was increased, and the small blood transfusions were given more frequently. The blood count gradually increased after this so that forty hours after the increase in liver dosage, the total white count was 5000, with 6 per cent myeloblasts, 30 per cent myelocytes, 9 per cent segmented neutrophilic forms, 7 per cent eosinophiles, 2 per cent basophiles, 32 per cent lymphocytes, and 14 per cent monocytes.

The bone marrow apparently regained its normal ability to maturate granulocytic neutrophiles, since, when the patient developed a severe acute systemic infection on Aug. 10, the white blood count rapidly rose to 36,600, with 95 per cent granulocytes. The infection was controlled by the use of sulfadiazine, without effect on the bone marrow, but he eventually died on Sept. 11, 1942, from liver failure. The icteric index gradually decreased from 285 to 200, and remained at approximately this level until death. Autopsy revealed severe yellow atrophy of the liver, marked degeneration of the corpus striatum of the brain, marked wasting of the striated muscles, and a normal bone marrow.

DISCUSSION

There are many reports of the use of sodium benzoate in the treatment of the rheumatic state, and more recently for the determination of hepatic function in the hippuric acid excretion test. Bryan[1] in 1925 stated "A review of the literature indicated that normal human beings may completely convert at least 25 grams of sodium benzoate to hippuric acid without toxic effect and with the appearance of only traces of free benzoic acid in the urine." Quick[2] felt that sodium benzoate was a safe drug in moderate amounts when he devised the hippuric acid excretion test in 1933. Snell and Magath[3] feel that the hippuric acid excretion test is a test of the detoxifying function of the liver and is based on the conjugation by the liver of amino-acetic acid and benzoic acid into hippuric acid, but mention no harmful effect from its use. Bartels[4] repeated the test three times on most of 148 cases with thyroid disease, without untoward effect. Hirschheimer[5] did 246 tests on pregnant women, some complicated and others uncomplicated, without ill effect. The *Journal of the American Medical Association*[6] stated, "There is no direct or indirect evidence to indicate that sodium benzoate has any toxic effect on the liver. The drug was formerly used for the treatment of rheumatic disease and has been given to human beings in doses as large as 25 to 60 grams a day without producing any harmful effect. Quick's hippuric acid test has been performed on many patients with extreme degrees of injury to the liver without any appreciable toxic effects or without depressing other hepatic functions; slight gastric irritation has been noted in a few cases, but these symptoms are of a temporary nature." Quastel and Wales[7] found that there was low excretion of hippuric acid after both oral and intravenous administration of sodium benzoate in schizophrenia, but mentioned no ill effects. Davies, Wales, and Hughes[8] were of the same opinion and mentioned seventy-five cases

without complications. Londe and Probstein[9] performed a modified hippuric acid test in many children without ill effect. Finkelman, Hora, Sherman, and Horwitt,[10] Campbell,[11] Vaccarro,[12] Snell and Plunkett,[13] Lipshutz,[14] Quick,[15] Haines, Magath, and Power,[16] and many others reported using this test in numerous cases, and none mentioned systemic reactions.

The absence of neutrophiles in the peripheral blood in this case, known by various names such as granulopenia, granulocytopenia, agranulocytosis, agranulocytic angina, leucopenia, or neutropenia, and which was described originally by Schultz[17] in Germany in 1922, and then by Lovett[18] in America in 1924, has not been described following ingestion of sodium benzoate. During the past twenty-one years, there have been numerous case reports and discussions, which have shown that with the increase in use of amidopyrine, arsphenamine, sulfonamides, and other substances depressing to the bone marrow, the incidence of granulocytopenia has increased. While a great many of these cases occur spontaneously, it is interesting to note that in most case histories there has been a preceding treatment of some type, by either a dentist or a physician. In Kracke's[19] series of 473 patients, nearly ¾ had been receiving treatment for some other disease for a variable period prior to the attack of granulocytopenia.

Granulocytopenia in the various races has been discussed, and it has been found to be rare in the colored race, occurring eight times in the 473 cases collected by Kracke.[19] A review of the literature further reveals that it occurs in a ratio of about two female patients to one male.

The cause of granulocytopenia has been discussed at great length. The first investigators directed their efforts on the hypothesis that the cause was some type of infection, because various organisms were usually cultured from the lesions in the throat and blood stream. Also, Fried and Dameshek[20] were able to produce a type of primary granulocytopenia in rabbits by the injection of *Salmonella suipestifer*. Kracke[19] doubts that Fried and Dameshek reproduced a true granulopenic picture, but produced the picture which is commonly seen following injections of any killed organism. The more recent work is directed toward the fact that granulocytopenia is a disease of the bone marrow, and that the basic picture is the disappearance of the neutrophiles from the peripheral blood, which, in turn, is followed by the invasion of any and every organ that is accessible by organisms. According to Jackson (Cecil's Text),[21] the eosinophiles disappear early and reappear late. The total number of lymphocytes usually is reduced. Pus does not form during this stage. Reznikoff[22] feels that the following four factors are important in causing this disease: fatigue, drugs, menstruation, and infection. In the thirteen cases studied by him, he found that fatigue due to excessive work, lack of sleep, and worry was more frequently encountered than any of the other etiologic conditions, and it was also his opinion that monocytosis is an evidence of good prognosis when it occurs early and is persistent.

While the majority of cases are due to toxic action of a drug on the bone marrow, a smaller group is due to the toxic effect of an infection on the bone marrow, as for instance, typhoid fever, influenza, and measles, which are associated with a leucopenia. There are probably three causes: (1) a primary form with fever, ulcerative mouth lesions, and agranulocytosis, (2) malignant granulocytopenia of bacterial origin, and (3) malignant granulocytopenia of toxic origin. Ralph U. Leser,[23] in discussing agranulocytic angina due to the sulfon-

amide drugs, suggests treatment by parenteral liver therapy and by mouth, if possible; repeated daily transfusions of 200 to 300 c.c.; pentnucleotides; a high caloric diet; good nursing; adequate cardiac and respiratory stimulation; withdrawal of all analgesic drugs containing the benzene ring, and also all the so-called barbiturates. He feels that adenine sulfate is quite valuable and that local treatment of the oral lesions is important. Ottenberg[24] used transfusions from donors who had received typhoid vaccine to stimulate leucocytosis in treating leucopenia. He reports only one case and believes that the cure was unquestionably due to the treatment. He feels that this is the result not of the actual number of leucocytes transfused, but to the passive transmission of the bone marrow-stimulating factor. Dameshek and Wolfson[25] treated two cases of severe agranulocytosis with sulfathiazole in addition to transfusions, pentose nucleotides, and liver extract, feeling that the recovery, at least in part, was due to the effect of the sulfonamide drug on the sepsis, which was almost certainly present.

TABLE II

TOTAL NUMBER OF WHITE CELLS OF EACH CLASS, AND TREATMENT

	W.B.C.	NEUTROPHILES	EOSINOPHILES	BASOPHILES	LYMPHS.	MONOS.	TREATMENT	
							TRANSFUSIONS	LIVER IN UNITS
1	5,850	3,510	176	0	1,872	292		
2	4,450	1,780	356	312	2,002	0		
3	1,600	0	64	16	976	544	250 c.c.	100 units intramus.
4	2,400	0	120	48	1,392	840	250 c.c. A.M. & 250 c.c. P.M.	200 units intramus.
5	2,650	0	106	26	1,590	928	250 c.c.	100 units intramus.
6	3,200	64	288	0	1,248	1,600	250 c.c.	100 units intramus.
7	4,700	188	235	94	3,478	705	250 c.c.	200 units intramus.
8	5,000	0	300	0	2,550	2,150	250 c.c.	200 units intramus.
9	5,000	2,250	350	100	1,600	700	250 c.c. A.M. & 250 c.c. P.M.	400 units intramus.
10	7,050	3,666	564	141	1,622	1,057	250 c.c.	200 units intramus.
11	7,350	2,866	515	294	3,014	661	250 c.c.	200 units intramus.
12	7,500	3,675	1,275	150	2,100	300	250 c.c.	200 units intramus.
13	8,300	3,652	747	83	2,739	1,079	250 c.c.	200 units intramus.
14	8,150	3,341	489	0	3,260	1,060	500 c.c.	400 units intramus.
15	6,500	3,185	390	65	2,015	845	250 c.c.	200 units intramus.
16	6,150	2,952	307	62	2,091	738	250 c.c.	100 units intramus.
17	8,350	5,146	83	166	2,905	0		100 units intramus.
18	11,100	5,550	666	222	3,219	1,443		100 units intramus.
19	14,700	10,290	295	147	3,528	441		100 units intramus.
20	10,200	6,120	510	204	2,856	510	500 c.c.	100 units intramus.
21	20,350	18,315	0	203	1,832	0	500 c.c.	
22	36,600	34,770	0	0	1,830	0	500 c.c.	
23	18,950	18,005	0	0	945	0	500 c.c.	
24	14,100						500 c.c.	
25	10,050	7,200	1,100	200	900	600		
26	9,950	6,732	495	198	1,832	693		
27	13,350	8,512	1,064	266	3,109	399		

Apparently the bone marrow varies in its appearance during the various stages of the disease, and there are at least two types of bone marrow seen in the early stages, one in which there is myeloid hypoplasia, and another in which there is arrest in maturation of the myeloid elements. From the literature one would assume that where myeloid hypoplasia exists, the prognosis is poor, whereas in those in which there is arrest of maturation of the myeloid elements, the outlook is better. Boyd[26] states "The bone-marrow varies in the fatal cases. As a rule it is aplastic and appears to be completely exhausted, as

is seen in experimental benzol poisoning. In others it is normal, and in still others it may be hyperplastic. In these latter cases there would appear to be a blockade of the marrow or a lack of maturation of the myelocytes, thus preventing the mature leucocytes from entering the circulation, a state of affairs comparable with what is found in pernicious anemia."

Kracke and Parker[27] in 1938 thoroughly reviewed the literature of drug therapy in relation to the neutropenic states, and cited eleven cases of granulocytopenia, nine with fatal termination resulting from the use of sulfanilamide. In all instances a large amount of the drug was administered over a relatively long period before the condition made its appearance. Rinkoff and Spring[28] in 1941 reported six cases illustrating the toxic effect of sulfonamides on the myeloid elements, three due to sulfanilamide, two to sulfapyridine, and one, a combination of sulfapyridine and neoprontosil. Three of their patients developed granulocytopenia within the first twenty-four hours or less of therapy, and three, after fourteen days or more of therapy. Three of these cases developing within twenty-four hours would mitigate against Kracke's experience that prolonged therapy with sulfanilamide was necessary to induce granulocytopenia.

The reactions occurring in our patient were probably of the so-called "anaphylactoid type." This term is used here to indicate a reaction closely resembling anaphylactic shock, but occurring after a chemical. The predominating symptoms of severe chest pain, extreme dyspnea, and orthopnea, early increase in blood pressure with later fall, rapid thready pulse, and shock, indicate the more common cardiac rather than pulmonary type of reaction. There apparently was no desensitization after the first reaction, as the second was as severe as the first. The increase in icteric index could be interpreted as the result of the anaphylactoid reaction, or as the direct result of chemical poisoning.

SUMMARY

This case presented various interesting problems. The patient apparently had a reaction following his first dose of sodium benzoate with some increase in his icteric index, probably due to further damage of the liver, and a second dose forty-eight hours later again caused a severe reaction and probably further increased the liver damage. The combination of the two doses produced an arrest of the maturation of the neutrophilic granulocytes in the bone marrow, but allowed the basophiles and eosinophiles to maturate. Another patient with acute hepatitis and an even higher icteric index had no reaction from either the first or second test, performed at the same time as the case being reported here.

Liver damage, or increase in damage to an already diseased liver, following sodium benzoate, must be extremely rare, as we have been unable to find any report of cases in the literature. The increase in jaundice and the increase in general debility of the patient could be interpreted as indicating further damage to the liver either as a direct result of the chemical or as a result of the reaction.

As to the bone marrow and peripheral blood, the situation was also peculiar in that it is customary for the eosinophiles and basophiles to disappear from the peripheral blood, and their maturation to be arrested along with the

other granulocytes, but in this case they appeared in normal to increased numbers in the peripheral blood, and apparently matured normally in the bone marrow. It is impossible to say what part, if any, the severely damaged liver played in the granulocytopenia, except that there was response to very large doses of liver. A point against the liver having any particular part is the fact that later there was a white count of 36,600, secondary to a severe septicemia, showing that the bone marrow could respond even though a badly damaged liver condition was still present. Further proof that the liver probably played little part was the fact that the bone marrow was normal at autopsy even though very few normal liver cells remained.

CONCLUSION

1. A colored male with marked yellow atrophy of the liver had a severe reaction on two occasions, approximately four hours after ingesting sodium benzoate, manifested by excruciating substernal pain, elevation of blood pressure, dyspnea, and orthopnea, followed by shock.

2. Three days after ingesting the second dose of sodium benzoate, the patient developed signs of systemic infection and the white blood count showed an absence of the neutrophilic granulocytes, but a normal to increased number of eosinophiles and basophiles.

3. The bone marrow showed arrest of maturation of the neutrophilic myeloid elements, but normal maturation of the eosinophiles and basophiles.

4. There was later a marked leucocytic response to a generalized septicemia, showing complete recovery of the bone marrow.

5. The icteric index increased from 220 to 275 after the first reaction, and to 285 after the second reaction. The cause of the increase in icteric index after the reactions remains undetermined.

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LABORATORY METHODS

GENERAL

THE EFFECT IN VITRO OF PROPYLENE GLYCOL ON ERYTHROCYTES*

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THE fading from the microscopic field of erythrocytes suspended in equal parts propylene glycol and water led to the use of this diluent for the enumeration of white blood cells.[1, 2] The red cells remained clearly visible for about three minutes in the white cell counting chamber after whole blood had been diluted with propylene glycol and distilled water. In five minutes the red cells had almost completely disappeared, remaining as vague "ghost" forms at room temperature (20° C.) for as long as ten days.

Equally striking macroscopic changes were observed in the test tube when blood was mixed with equal parts propylene glycol and water. In three to five minutes after mixing, the initially turbid solution became clear, suggesting complete hemolysis. Earlier investigators have reported the hemolytic action of glycols, but this explanation of the disappearance of erythrocytes is refuted in the present report by dark-field microscopic examinations which showed the red cells still present in normal numbers in the apparently hemolyzed preparation. In higher concentrations of propylene glycol or in the presence of temperatures over 20° C., the faded red blood cells spontaneously reappeared. These observations led to the present study of the mode of action of propylene glycol, as hemolysis could no longer be considered the mechanism responsible for the initial fading of the red cells.

Bachem[3] in 1911 observed hemoglobinuria in animals following the intravenous injection of ethylene glycol, while the initial observation regarding the action in vitro of glycols on blood was made by Von Oettingen and Jirouch in 1931.[4] The hemolytic action of propylene glycol in vitro was studied by Lehman and Newman[5] in 1937 by a procedure similar to testing for red blood cell fragility. One part of rabbit blood was added to ten parts of mixed propylene glycol and water in which the glycol content did not exceed fifty per cent. Propylene glycol produced immediate hemolysis in all aqueous concentrations.

In commenting on these observations, Weatherby and Haag[6] concluded that aqueous solutions of propylene glycol produced rapid and complete hemolysis in a manner similar to that of distilled water. They assumed that the

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glycol entered the red blood cell membrane so rapidly that it exerted no appreciable osmotic effect on the cell. This explanation had been offered by Johnson, Carlson, and Johnson[7] for the apparently similar type of hemolytic phenomenon occurring with aqueous solutions of glycerol or urea.

Behavior of Erythrocytes in Mixtures of Propylene Glycol and Distilled Water.—Procedure.—Propylene glycol and distilled water were mixed in various proportions in a series of test tubes maintained at 0°, 20°, 37°, and 50° C. The first tube of each series contained only distilled water, the second, 10 per cent glycol, and the succeeding tubes contained increasing amounts of propylene in 10 per cent increments. One part of human venous blood was added to twenty parts of each solution; the tubes were inverted to mix, corked, and observed at intervals for a period of four days.

TABLE I

TIME OF FADING OF ERYTHROCYTES FROM MICROSCOPIC FIELD IN AQUEOUS CONCENTRATIONS OF PROPYLENE GLYCOL; COINCIDENT WITH CHANGE OF SOLUTION FROM TURBID TO CLEAR

PER CENT PROPYLENE GLYCOL IN WATER	TEMPERATURE			
	0° C.	20° C.	37° C.	50° C.
100	½ min.	—————immediate—————		
90	1½ min.	1 min.	½ min.	½ min.
80	4 min.	2 min.	¾ min.	½ min.
70	20 min.	2½ min.	1 min.	1 min.
60	22 min.	3 min.	1 min.	1 min.
50	25 min.	3 min.	1½ min.	1½ min.
40	25 min.	8 min.	1½ min.	1½ min.
30	25 min.	150 min.	1½ min.	1½ min.
20	22 min.	8 min.	1½ min.	1½ min.
10	5 min.	2 min.	1 min.	1 min.
0	2 min.	1½ min.	1 min.	1 min.

Macroscopic Observations.—Three stages in the gross appearance of the blood-propylene glycol-aqueous mixtures were observed. The solutions were first turbid. This stage was followed by a gradual clearing until the solutions became transparent, suggesting complete hemolysis. The time required for the transition from turbidity to translucency (Table I) depended upon the temperature and glycol concentrations; it occurred more rapidly at higher temperatures and at both extremes of propylene glycol concentration. The longest interval was noted with 30 per cent glycol at 20° C. The third and final stage was characterized by the redevelopment of a turbid solution due to the formation of a reddish-brown flocculent precipitate. Von Oettingen and Jirouch[4] reported the formation of a similar precipitate with glycols although they did not use propylene glycol. As shown in Table II, the final precipitate developed only with higher temperatures and with higher concentrations of glycol; the time required for beginning precipitation varied with the temperature and glycol content. Except with one hundred per cent glycol, the formation of the precipitate was a gradual process. In the course of the reaction progressively larger amounts of precipitate were obtained as measured by centrifugation until an end point was reached, after which the amount of precipitate decreased and the supernatant fluid became clear. In later studies similar results

were obtained by measuring the amounts of precipitate by means of a photoelectric colorimeter.

A similar precipitate developed when erythrocytes, thrice washed and resuspended in saline, were added to 60 per cent and higher concentrations of propylene glycol in water. Cell-free plasma also precipitated when added to the same concentration of propylene glycol. From these observations, it appeared that the reaction giving rise to flocculation and precipitation might be one affecting all blood proteins and might not be limited to the erythrocytes.

Microscopic Observations.—The mixture of blood in 80 per cent propylene glycol in water was selected for detailed microscopic observation, because at this concentration it was possible to observe the three phases of the reaction within a relatively short time. As viewed under oil immersion, the erythrocytes faded in two minutes although many faintly visible and nonrefractile red cell forms remained; these were brought out more clearly by reducing the light or by making minor adjustments in the condenser. After about thirty minutes, the cells gradually became more distinct; with average illumination one obtained the impression that the red cells had "reappeared." With further time, many erythrocytes developed hairlike projections at right angles to the cell wall; each was associated with the development of an increasing amount of granular, extracellular debris. Spontaneous agglutination of these abnormal cells then occurred, giving rise to the macroscopic appearance of turbidity and flocculation. The individual cells of these agglutinated masses finally disintegrated, leaving only debris, this stage corresponding to the end point of macroscopic precipitation.

Similar microscopic changes in the erythrocytes occurred in other mixtures containing over sixty per cent propylene glycol, except that the rate of the reaction varied in proportion to the glycol content. In a concentration of 60 per cent glycol at 20° C., a few red cells remained refractile and clearly visible at the end of seven days; the number of such cells was apparently not sufficient to result in the gross appearance of turbidity. Agglutination and flocculation were not observed in this tube and did not occur in other tubes containing less amounts of propylene glycol. A microscopic study of the "ghost" forms in lower concentrations was not undertaken because of their relatively poor visibility as viewed in the conventional microscope.

With 30 per cent glycol and 70 per cent water at 20° C., the red cells, although enlarged, remained clearly visible for approximately two and one-half hours before fading.

Dark-Field Observations.—Blood was added to various concentrations of propylene glycol and examined immediately under the dark-field microscope. In an aqueous solution of 70 per cent propylene glycol at 20° C., the red cells became uniformly shrunken and rapidly crenated. Agglutination soon followed, and by the end of thirty minutes all erythrocytes had disintegrated. Red cells in 50 per cent propylene glycol appeared normal in size and contour two minutes after mixing. The cells gradually became shrunken and crenated; with the passage of time, many became agglutinated and some possessed spicules extending at right angles from the cell wall. Although distorted in form, the erythrocytes remained clearly visible for as long as ten days. With decreasing

concentrations of propylene glycol, a progressive swelling of erythrocytes was noted, eventually leading to the disruption of the cells.

Behavior of Erythrocytes in Mixtures of Propylene Glycol and Physiologic Saline.—Lehman and Newman[5] in 1937 produced hemoglobinuria in animals by the intravenous injection of a 33.3 per cent solution of propylene glycol in saline. They concluded that the hemoglobinuria resulted from the hemolysis of erythrocytes and was due to the asmotic action of glycol on the cells at the site of the injection. The authors further mixed one part rabbit blood with ten parts propylene glycol in saline solutions and observed no hemolysis in concentrations of 40 per cent propylene glycol and below at the end of three hours; in 40 per cent glycol, partial hemolysis was present at twenty-four hours. Immediate hemolysis was produced under the same conditions and concentrations by propylene glycol and water.

Similar findings were reported by Weatherby and Haag[6] with human blood in various concentrations of propylene glycol and physiologic saline. They felt that the absence of hemolysis in saline solutions of propylene glycol in this range was to be expected as a 30 per cent concentration was essentially an isotonic medium.

TABLE II

TIME OF "REAPPEARANCE" OF ERYTHROCYTES IN MICROSCOPIC FIELD IN AQUEOUS CONCENTRATIONS OF PROPYLENE GLYCOL; COINCIDENT WITH CHANGE OF SOLUTION FROM CLEAR TO TURBID AND APPEARANCE OF GROSS PRECIPITATE

PER CENT PROPYLENE GLYCOL IN WATER	TEMPERATURE			
	0° C.	20° C.	37° C.	50° C.
100	1 min.	———	immediate ———	
90	1,200 min.	4 min.	3 min.	1 min.
80		40 min.	5 min.	2 min.
70		600 min.	20 min.	3½ min.
60			150 min.	4½ min.
50			2,400 min.	5 min.
40				10 min.
30				25 min.
20				80 min.
10				180 min.
0				------

Present observations on whole human blood in saline concentrations of propylene glycol confirm the reports of previous authors. Twenty-four hours were required for the apparent hemolysis of solutions containing 40 per cent glycol and forty hours for 30 per cent solutions. The red cells remained intact and the solutions remained turbid for as long as seven days in concentrations of propylene glycol below 30 per cent. In amounts of 60 per cent glycol and above, the clear solutions later became turbid and developed a reddish brown precipitate. This phenomenon occurred more rapidly in higher concentrations of propylene glycol and in all respects appeared similar to the reaction occurring with aqueous solutions. At a time when the gross appearance of the solution suggested complete hemolysis, microscopic examination revealed the faded "ghost" forms previously described, but the red cells remained clearly visible and in normal numbers when observed under dark-field illumination.

The Action of Other Glycols on Erythrocytes in Vitro.—The action of ethylene glycol and its derivatives on blood was studied by Von Oettingen and Jirouch.[4] They added 1 c.c. of various glycols to 5 c.c. fresh dog blood or beef serum; ethylene and diethylene glycol failed to precipitate whereas ethyl ethylene glycol acetate, one to four dioxan and butyl ethylene glycol caused marked precipitation.

In the present study aqueous solutions of ethylene and triethylene glycol with human blood were observed in the proportions previously described. Except with 30 per cent concentrations where the time required for the solutions to clear was prolonged, all other concentrations of both glycols resulted in the gross appearance of hemolysis within fifteen minutes. The speed of the clearing of the reaction was more prolonged at room temperature than was the case with propylene glycol. In higher concentrations, both ethylene and triethylene glycol produced a similar type of reddish-brown precipitate.

Discussion.—Erythrocytes are visible in the conventional bright field microscope because of their ability to refract light. This in turn is dependent upon a difference in the relative densities of the cells and the surrounding medium. When erythrocytes are suspended in solutions of over 30 per cent propylene glycol in water, the density of the red cell apparently approaches that of the solution. Light rays may then pass from one to the other without significant bending, with the result that the erythrocytes become relatively invisible in the conventional microscope, although clearly outlined in the dark field, and the gross appearance of the suspension changes from one of turbidity to clearness.

The "fading" of erythrocytes in the presence of less than 30 per cent propylene glycol in water appears to be primarily the result of hemolysis, as evidenced by progressive swelling of the cells prior to their disappearance. The rate of "fading" is, furthermore, roughly proportional to the hypotonicity of the mixture, and relatively few intact cells are seen when the clear solutions are viewed in the dark-field microscope.

Thirty per cent propylene glycol and 70 per cent water apparently represent a critical glycol concentration, above which the red cells are affected primarily by glycol and secondarily by water, and below which primarily by water and secondarily by the glycol content. The marked delay in the "fading" of erythrocytes in this concentration at 20° C. is very likely due to the relatively ineffective glycol action at this temperature and dilution and the mild hypotonicity of the solution.

The spontaneous reappearance of red cells at higher temperatures and glycol concentrations is presumably the result of an increase in the intracellular versus the extracellular density and is most probably associated with the coagulation or precipitation or other alteration of cellular proteins, a process which finally leads to cell destruction and the formation of a dark granular precipitate. Alteration of cell-free plasma also occurs in the presence of higher concentrations of propylene glycol, resulting in precipitate formation; this suggests that the physiochemical reaction produced by propylene glycol is not one specifically affecting erythrocytes.

In concentrations above 30 per cent propylene glycol, erythrocytes behave similarly whether suspended in glycol-aqueous or glycol-saline solutions. Al-

though the "fading," "reappearance," and final disintegration of cells develop at different rates with alterations of the temperature and glycol concentration, at a given temperature and content of glycol the effects occur much more slowly in the presence of saline. The retardation becomes more marked as the glycol percentage is decreased and that of saline increased; for instance, at 40 per cent and 30 per cent glycol in saline, twenty-four and forty hours are required for the "fading" of erythrocytes from the bright field.

There is a fundamental difference in the behavior of red cells in concentrations below 30 per cent propylene glycol according to whether the solutions contain saline or water. In the saline, the red cells remain intact and clearly visible for many days. The presence of more than 70 per cent physiologic saline appears to protect the red cells from the action of glycol. In the water, the erythrocytes "fade" roughly in proportion to the hypotonicity of the solution.

Ethylene and triethylene glycol exert essentially similar effects on erythrocytes as produced by propylene glycol.

Summary.—1. Observations are reported on erythrocytes suspended in aqueous and saline concentrations of propylene and other glycols.

2. The erythrocytes were found at first to disappear from the microscopic bright field but not from the dark field, coincident with the apparent gross "hemolysis" of the blood-glycol solution.

3. Evidence is offered refuting the suggestion that hemolysis was the cause of the initial fading and disappearance of erythrocytes.

4. In time, depending on temperature and glycol concentration, the erythrocytes reappeared in the microscopic bright field.

5. The nature of the reaction between glycols and erythrocytes is considered.

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THE EFFECT IN VITRO OF PROPYLENE GLYCOL ON LEUCOCYTES*

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THE observation that leucocytes remain intact and clearly visible when suspended in equal parts of propylene glycol and distilled water led one of us (T. G. R.) to use this solution as a white blood cell diluting fluid.[1, 2] In comparison with the standard 2 per cent acetic acid diluting fluid, the glycol diluent possesses several advantages inherent in its greater viscosity, facilitating filling the counting chamber and decreasing the subsequent rate of evaporation. The incorporation of acid and basic stains, readily soluble in propylene glycol, further permits the differential staining of eosinophils, polymorphonuclear and mononuclear cells in the counting chamber. It is therefore possible to obtain a differential white cell count in the counting chamber on the same sample of blood used to enumerate the total leucocytes. Mononuclear cells include both lymphocytes, monocytes, and nonsegmented members of the polymorphonuclear series. Basophils are not differentiated from other polymorphonuclear cells. Eosinophils are stained characteristically, thus enabling their direct enumeration free from the errors of distribution inherent in the procedure of preparing and counting the Wright-stained film. The performance of a counting chamber differential in conjunction with the differential white count from the stained film gave additional evidence as to the identity of "smudges" encountered occasionally in films. The blood of twenty normal subjects was studied in the preliminary report, and this diluent yielded accurate total leucocyte counts when compared with parallel determinations with 2 per cent acetic acid diluting fluid. Counting chamber differential counts were also in good agreement with differential counts performed from the Wright-stained film.

The further use of stains dissolved in 50 per cent aqueous propylene glycol has confirmed the conclusions drawn in the preliminary study. It has been observed that blood diluted with this fluid may be allowed to stand in the pipette overnight without significant change in the total white count or impairment in the staining qualities of the leucocytes.

I. *Observations on Normal White Blood Cells.*—In a further study of leucocytes, one part of whole human venous blood was added to twenty parts of propylene glycol in distilled water. The mixtures were placed in a series of ten tubes, the glycol content of which varied by increments of 10 per cent; the first tube contained 10 per cent glycol and 90 per cent water, the final tube, 100 per cent propylene glycol. Each tube was corked, maintained at 20° C., and, prior to withdrawing samples for counting chamber study, was shaken gently. Observations were made on fresh samples at intervals of thirty minutes for the first three hours and every twelve hours thereafter for sixty

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hours. White cells were nonmotile in all samples. Eosinophils were not identified in the absence of stains, although mononuclears could be differentiated from polymorphonuclear leucocytes.

In a concentration of 60 per cent propylene glycol in distilled water the white cells remained for as long as sixty hours without apparent change. This concentration of glycol was found to represent a critical point, for with lesser amounts of glycol, the white blood cells became progressively enlarged, irregular, and less clearly outlined; the rate and degree of these changes varied inversely with the content of propylene glycol. At 50 per cent, 40 per cent, and 30 per cent propylene glycol, the mononuclear cells were the first to become enlarged and irregular, followed by similar changes in the polymorphonuclear leucocytes; the changes in both types of cells occurred more rapidly with progressive dilutions. Cells in 50 per cent solutions showed slight changes, but remained clearly visible and at a constant numerical level for as long as sixty hours. In 40 per cent solutions, the leucocytes showed more marked abnormalities in their size, shape, and refractility; at the end of two hours, distortion and fading interfered with their enumeration. In 30 per cent and lower concentrations of propylene glycol, the above changes occurred immediately and were followed by a tendency of the leucocytes to agglutinate. The initial white cell count at this concentration was below normal and with serial determinations continued to fall.

At concentrations above 60 per cent propylene glycol, the leucocytes became smaller in size. In a 70 per cent solution, the white cells remained clearly visible for two hours; after this time the gradual "reappearance" of erythrocytes interfered with the enumeration of leucocytes. At concentrations above 70 per cent, this "reappearance phenomenon" occurred too rapidly to allow the initial enumeration of white cells, although the latter remained visible in concentrations of 90 per cent and 100 per cent propylene glycol. The phenomenon of "reappearance" of erythrocytes in higher glycol concentrations has been described.[3]

In an attempt to determine the range of propylene glycol concentrations in which the size and shape of white cells remained unaltered, blood was added similarly to mixtures of 58 per cent and 62 per cent glycol in water. In the lower concentration mononuclear cells became slightly enlarged at the end of thirty minutes and polymorphonuclear cells became distorted at the end of two hours. In 62 per cent glycol all white blood cells became uniformly small but remained refractile and clearly visible for sixty hours.

Differential Staining.—As previously described, eosin and methylene blue each as a 0.1 per cent aqueous solution in 50 per cent propylene glycol produced a satisfactory stain for the leucocytes with a minimum of distortion. Blood placed in such a solution was studied under high magnification. Within a five-minute period the granules of the eosinophil stained a bright red. The capsule became progressively and uniformly enlarged until it finally appeared as an eccentric swelling. The intervening space between the capsule and the cytoplasm remained clear except for an occasional granule observed on the capsular rim. The eccentric arc-like enlargement of the capsule frequently reached a size twice that of the parent eosinophil; it was occasionally seen to rupture

although this did not occur within the first thirty minutes. After rupture of the membrane the cell remained intact, spherical in outline, and surrounded by a small concentric capsular ring.

Polymorphonuclear neutrophils in 50 per cent propylene glycol showed similar but less frequent and extensive changes. Capsular swelling was usually concentric and only detectable under oil immersion. Eccentric swelling was seen rarely in normal blood nor was rupture of the capsule observed. As with eosinophils, capsular swelling of neutrophils occurred more readily at lower concentrations of glycol and was not detected in 60 per cent propylene glycol in water.

Mononuclear cells did not show a capsular swelling. However, a uniform enlargement of the cell was seen in 50 per cent but not in 60 per cent propylene glycol.

All leucocytes were more deeply stained in concentrations of propylene glycol below 50 per cent, but the distortion of the cells in these aqueous concentrations did not favor their use. The staining qualities were distinctly inferior at 55 per cent and 60 per cent propylene; at higher strengths the cells failed to stain.

TABLE I

COMPARISON OF THE TOTAL LEUCOCYTE COUNTS USING 2 PER CENT ACETIC ACID AND PROPYLENE GLYCOL-AQUEOUS STAINS

PATIENT	DIAGNOSIS	TOTAL WHITE CELL COUNT	
		PROPYLENE GLYCOL-AQUEOUS STAIN	2 PER CENT ACETIC ACID
L. H.	Lymphoblastic Leucemia	108,000	102,000
E. M.	Lymphoblastic Leucemia	127,000	123,000
F. T.	Lymphoblastic Leucemia	300	300
F. H.	Chronic Lymphatic Leucemia	31,000	27,000
W. G.	Lymphoblastoma	35,800	33,850
U. S.	Myelogenous Leucemia	319,000	292,000
M. S.	Myelogenous Leucemia	282,500	266,500
W. M.	Myelogenous Leucemia	45,000	40,500
F. M.	Myelogenous Leucemia	130,000	130,000
B. B.	Monoblastic Leucemia	170,000	195,000
F. S.	Monocytic Leucemia	92,350	87,500
W. B.	Histio-monocytic Leucemia	32,500	35,000
E. P.	Aleucemic Leucemia	2,900	3,500

The propylene glycol diluent consisted of equal parts of the two stock solutions. Maximum staining qualities were observed when the diluent was prepared from the stock each day.

Solution I
Methylene blue 0.1 Gm.
Propylene glycol 100 c.c.

Solution II
Eosin B 0.1 Gm.
Distilled water 100 c.c.

II. *Observations on Abnormal White Blood Cells.*—It became of interest to study the blood of patients with leucemia and with conditions characterized by the presence of abnormal or immature leucocytes. Table I records parallel total white blood cell determinations made by the standard 2 per cent acetic acid diluent and with the propylene glycol aqueous stain.

In the case of L. H. in the five days following irradiation therapy, the total leucocyte count increased from 6,700 to over 100,000 per cmm.; and a differential count made with the Wright-stained cover slip method revealed 92 per cent "smudges." Despite the apparent fragility of the white cells, the total leucocytes determined with propylene glycol agreed closely with that obtained

with 2 per cent acetic acid. The differential count from the Wright-stained cover slip revealed 3 per cent polymorphonuclear cells, 1 per cent eosinophils, 4 per cent large lymphocytes, and 92 per cent "smudges." A parallel counting chamber differential count identified the "smudge" forms as a mononuclear type cell with a differential showing 4 per cent polymorphonuclear cells, 1 per cent eosinophils, and 95 per cent mononuclear cells.

Propylene glycol and water in equal parts appears to be a satisfactory diluent for counting abnormal white blood cells in leucemia.

Discussion.—Enlargement of the capsules of eosinophils and polymorphonuclear leucocytes and a uniform enlargement of mononuclear cells occur in the presence of equal parts propylene glycol and water. This does not invalidate the use of this diluent for determining the total white cell count with pathologic cells as encountered in leucemia.

Satisfactory differential staining of leucocytes with eosin and methylene blue occurs only when the diluent consists of equal parts of propylene glycol and water. Optimum staining with minimum cell distortion occurs in 50 per cent propylene glycol in water, for with lower concentrations the cells become distorted in outline and visibility despite adequate staining. With 60 per cent propylene glycol staining is unsatisfactory, although no change in the size of the cells occurs for long periods of time.

Summary.—1. A white blood cell diluting fluid consisting of eosin 0.1 per cent and methylene blue 0.1 per cent dissolved in equal parts propylene glycol and water has been found to give accurate leucocyte counts in normal and abnormal blood.

2. This diluent possesses certain technical advantages over the use of the standard acetic acid diluting fluid.

3. The effect of various aqueous concentrations of propylene glycol on leucocytes in vitro has been described.

We wish to acknowledge the technical assistance of Elizabeth Barber Gibson and the valuable suggestions of Don E. Francke.

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A STUDY OF THE PAPPENHEIM STAIN—A STABLE MODIFICATION*

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PAPPENHEIM'S compound stain could be a useful aid to the bacteriologist for the examination of bacteria in pus if it were more stable. The differential qualities and the higher percentage of positive findings make further attempts at increasing its stability worth while.

Pappenheim[1] prepared his stain from solid or concentrated aqueous solutions of methyl green and pyronine in a study of lymphocytes and plasma cells. Unna[4] preferred a stock reagent of the two dyes in alcohol-glycerin solution with 0.5 per cent phenol added. Krzysztalowicz[5] employed 2 per cent phenol in the Unna reagent to decrease staining reaction time of gonococci in smears of exudates. Saathoff[6] used higher concentrations of pyronine and alcohol as well as 2 per cent phenol in his stain for gonococci in pus smears. Conn et al.[7] suggested the use of pyronin "B" in place of pyronin "G." Pappenheim[3] and Scudder[8] advocated the use of the Pappenheim reagent as a counterstain in the Gram reaction. Walton[9] suggested the use of methyl alcohol in place of ethyl alcohol.

Our previous efforts to employ the Pappenheim stain or its modifications were disappointing due principally because of the instability of the methyl green and the variation of dyes on the market. Freshly prepared products were satisfactory in our hands, but when reagents were given to others, indifferent results were obtained by them. Several experiences in our attempts to prepare a satisfactory Pappenheim stain may be of sufficient interest to record here.

A 1 per cent solution of methyl green in distilled water and a 1 per cent solution of methyl green in 2 per cent aqueous phenol were prepared and tested using the filter paper spot test.†

The methyl green in distilled water dissolved completely, and the spot formed on filter paper was green with a distinct blue or purple center. The methyl green in 2 per cent aqueous phenol, however, yielded a precipitate, and the supernatant fluid produced a green spot on filter paper without a trace of purple or violet. The precipitate formed in aqueous phenol was crystal violet.

A similar precipitate may be produced by adding phenol to aqueous crystal violet solutions, the degree of precipitation being a function of the concentration of phenol.

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†The filter paper spot test was employed by Pappenheim[1] as an aid in preparing multiple stain reagents. A drop of stain was placed on a filter paper and allowed to spread. The periphery of the spot showed the relative amount of green in the stain, while the center showed the amount of red. Using this test one could estimate the effective concentration of both methyl green and pyronine or the concentration of crystal violet impurity in the methyl green stock solutions. One may determine roughly how much to dilute either constituent with the other or with water. This test has been useful, but not all dye combinations showing unequal diffusion through filter paper can be used in Pappenheim compound stains with success.

Pappenheim reagents prepared as described by Saathoff and by Walton (both modifications contained 2 per cent phenol) produced a precipitate of crystal violet which formed a gummy residue on the glassware and filter. Hence, the phenol served to remove by precipitation the crystal violet which was contained as an impurity in the commercial methyl green dye.*

Stain reagents prepared according to Walton's formula, or prepared from stock aqueous solutions of purified methyl green and pyronine, gave suitable stains when freshly prepared. In our hands, however, the methyl green continued to split off crystal violet. Walton[9] reasoned that methanol, substituted for ethanol, would suppress the dissociation of the loosely bound methyl group of methyl green and retard the formation of crystal violet. This might be the case if the methyl green were dissolved in aqueous methyl alcohol alone, but it would seem that inhibition of the crystal violet formation would be very slight in the Walton formula, since the phenol present would precipitate the crystal violet and encourage the reaction (methyl green $\rightarrow$ crystal violet $\downarrow$ + CH_3OH) to go to completion. To employ a reagent which would not precipitate the crystal violet would impair the differential character of the stain, as crystal violet stained gonococci and leucocytes and debris with equal intensity.

Pyronine seemed to have an unusual affinity for the Neisserian organisms. It was stable under a wide range of conditions and its use in the stain offered no great difficulty. The need was for a stable dye of contrasting color which would stain the nucleus of a leucocyte intensely, the cytoplasm of the cell poorly, and the intracellular bacteria still less or not at all. In looking for dyes that might take the place of methyl green, we examined the blue and green basic dyes that are commonly employed in bacteriologic laboratories. Two such dyes were brilliant green and malachite green. Both dyes when used in combination with pyronine gave a filter paper spot test as good as that obtained with a good, fresh methyl green reagent.† Although both of these basic green dyes were described by Pappenheim[2] as unsuitable for combination with pyronine in his differential stain, it has been our experience that either may be utilized satisfactorily for the production of a stable Pappenheim-type stain. It has seemed that malachite green produced a slightly better product, but either dye gave satisfactory color contrast.

The following formula is suggested as a guide in the preparation of a stable modification of the Pappenheim reagent giving good differential stains:

1 per cent aqueous malachite green (oxalate, certified "NMg-3," dye content 75 per cent ---------- 1.2 c.c.
0.5 per cent aqueous pyronine G (certified "NP-7," dye content high, but not specified on label) or pyronine 2G (Coleman and Bell) -- 3 c.c.
Distilled water ---------- 20 c.c.

Prepare stock solution of malachite green in distilled water two days before use and filter. Stain from thirty seconds to three minutes. Wash with water.

*Conn[7] suggested that methyl green may be extracted with chloroform or amyl alcohol to remove the crystal violet impurity. Chloroform extraction has markedly improved the lot of methyl green with which we worked.

†Walton kindly supplied a sample of his reagent.

A good stain is produced by a delicate balance of the concentrations of the two dyes and of the time and temperature of their reaction on the tissues. The two dyes (the green and the red) must be balanced in respect to their affinity to the tissue and their color intensity. Thick smears stained deeper than thin smears, but differentiation was usually better in the thick portions of the preparations. The time of staining was important, for in overstained smears gonococci were dark, having taken some of the green color, and the contrast was poorer than in preparations stained for shorter periods.

It is suggested, in view of the variations in dye content of products on the market and in view of the preference of individuals using the stains, that stock solutions of each of the two dyes be prepared and several reagents be formulated in test tubes for trial, varying the ratio of the dyes until a product is obtained which gives a differentiation and contrast satisfactory to the user. The best reagent may then be prepared in larger volume and stored for use.

It should be noted especially that buffer salts of pH 4.7 or more precipitated and decolorized dilute solutions of brilliant green and malachite green, a change that did not take place at pH 4.5 or less. The tap water (pH 8.2) in our laboratory caused a precipitation and fading of these dyes. Some specimens of "distilled" water likewise caused slight changes in color intensity, but tests indicated that these samples were contaminated with alkaline substances carried over in the distillation. Dilute solutions of the dyes in distilled water of a good quality did not fade. It is possible that Pappenheim may have encountered similar conditions in his trial of these two dyes.

Because of the effect of alkaline salts on brilliant green and malachite green, aqueous solutions have been used employing *distilled* water. It has not been found necessary to incorporate glycerol, phenol, ethanol, or methanol in this modified stain. Pyrex glassware has been used for storage of the stock solutions to avoid the alkali of ordinary glass containers.

Freshly prepared solutions of malachite green may stain more intensely than those two or more days old. Aside from this initial change, stain reagents and stock solutions of the dyes have shown no depreciation in staining qualities during two years of storage at laboratory temperature on a shelf exposed to daylight. New stain reagents prepared from old or new stock solutions of malachite green and pyronine G were indistinguishable from old stain reagents.

Several serious limitations of the Pappenheim stain have been noted. Very thin smears were apt to be faintly colored even with a more extensive period of staining. Although these could still be examined microscopically and contrast was sufficient for observation of phagocytized organisms, they were more difficult to observe. The use of heavier smears (for which the Pappenheim stain is eminently suited) should remedy this situation.

A second limitation of the stain is less serious. The smears are best stained when treated individually. The separate handling of the slide preparations is offset by the simplicity and the rapidity of the staining procedure.

We can confirm Walton's statement that positive reports may result more frequently from the use of this stain in examination of gonorrheal exudates than from the exclusive use of the Gram stain. In some instances we have found typical organisms with relative ease in a Pappenheim-stained preparation, whereas the Gram-stained slide showed numerous leucocytes or much debris with-

out distinguishable bacteria of any kind. This was particularly true in heavy prostatic smears or in specimens containing considerable blood. We do not feel able to differentiate between gram-positive and gram-negative diplococci using the Pappenheim stain alone. We prefer to employ the reagent as a counterstain for the Gram reaction (as suggested by Pappenheim[3] and more recently by Scudder[8]), although this decreased the brilliance of the stain of the bacteria in the smear and it was necessary to shorten materially the staining time of the Pappenheim reagent.

The stain is useful in the examination of pus aspirated from chancroidal buboes, since the occasional *H. ducreyi* is so much more readily seen than with the Gram stain. Cultures of the Ducrey bacillus in the clotted rabbit blood described by Teague and Deibert[10] are likewise more easily examined when stained by the Pappenheim stain. The *young* Ducrey organisms stain intensely with the pyronine against a green background.

Many bacteriologists doubtless remember their first encounter with the Gram reaction and the dissatisfaction expressed by beginning students in bacteriology when first attempting to employ the stain. Experience and judgment are just as necessary in employing and interpreting results obtained with the Pappenheim stain. It is not believed that a stable Pappenheim-type stain will replace the Gram stain in routine microscopic examination of gonorrheal exudates, but a dependable product would undoubtedly be more generally employed to augment the staining procedures now used.

Summary.—A stain of the Pappenheim bicolor type which is stable and which gives as good differentiation as the original freshly prepared methyl green pyronine reagent is described.

The stain is of special value in the examination of bloody specimens, heavy smears of exudates, and blood clot cultures of chancroidal pus. It should be a valuable supplement to the Gram stain.

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ON THE CARE OF HUMAN SPERM*

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THE early reports concerning in vitro care of sperm were ubiquitous and inadequate. Since the study of sperm physiology and vitality has more recently been undertaken, their per se preservation has become fundamental. Furthermore, lack of ready availability of human sperm makes it more imperative that any test undertaken gives the desired results without repetition.

If the desired test is for survival time of spermatozoa or for artificial insemination, the donor should provide the ejaculate while at the office. The ejaculate should be collected directly into a chemically clean, sterile receptacle. A calibrated centrifuge tube serves well.

THE EFFECT OF RUBBER, OF BRASS, AND OF COPPER

Human ejaculates for study purposes are still frequently and erroneously collected from condoms. Rubber, even on short time exposure, decreases markedly the duration of motility of spermatozoa. Brown and Gamble (1943) report the results of several tests in which the ejaculate was divided, the one fraction used as a control and the other exposed to rubber, i.e., condoms and eye dropper bulbs. Some of the condoms had been thoroughly washed in tap water to remove the powder; others were used as packed. The age of spermatozoa exposed for only five minutes was reduced to 60 to 70 per cent of control time; with constant exposure, the spermatozoa had only 25 per cent of the control life span. The time difference with exposure to the unwashed condom was negligible. Constant exposure to brass, which may be encountered in lids, caps, etc., placed on a vial containing the ejaculate, decreased the longevity to approximately 30 per cent of the control. Copper essentially duplicated this effect.

THE EFFECT OF AIR AND TEMPERATURE

Several investigators have reported that air affects spermatozoa variously. In a series of experiments Brown and Gamble (1943) determined that there was no appreciable difference between the duration of motility of the control and sperm which had air excluded by an overlay of mineral oil. The effect of temperature, on the other hand, is pronounced. The average duration of locomotion in 14 experiments with an initial average spermicidal time of approximately one hundred minutes with 2.8 per cent potassium acid phthalate solution was 20 to 25 hours at body temperature (37° C.), 70 to 75 hours at

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†The author has observed several instances in which a leucocyte had ingested an active spermatozoon; pronounced flagellation ensued and continued for several minutes.

room temperature (approximately 27° C.), and 140 to 150 hours at refrigerator temperature (5° C.). The 2.8 per cent phthalate was mixed with an equal amount of semen, and the immobilization time (spermicidal time) was noted (Brown and Gamble, 1940, 1941). This was repeated at convenient intervals until the spermicidal time reached two to seven minutes; this was called the aging time or longevity (Brown and Gamble, 1941, 1943).

EFFECT OF VAGINAL SECRETION, URINE, PUS, AND BACTERIA

Brown (1943) determined that the longevity time of spermatozoa in a mixture of equal parts semen and vaginal secretion was reduced to 55 to 60 per cent of control time. Spermatozoa from the postcoital vagina, being constantly in contact with intermixed secretion, are inferior to the control. Urine which was normal by routine laboratory test reduced the longevity time to about 60 per cent of control time; diabetic urine (3+), to 80 per cent; and icteric urine (3+), to about 45 per cent (Brown, 1943). Pus from urethritis, etc., decreased the longevity variably depending on the degree of purulency. Pus cells may ingest the spermatozoa (3), and fragments and cells at times adhere to the flagellum, etc., of the spermatozoon, thereby decreasing its activity. Spermatozoa contaminated with bacteria invariably have a decreased longevity, probably from toxic metabolites. Motile bacilli may also be confusing to the microscopist when using low power oblique dark-field illumination.

ESSENTIAL CONTROLS

Because of the pronounced variation of spermatozoal vitality as determined by spermicidal times (Brown and Gamble, 1940, 1941, 1943), controls to determine the vitality and aging process of spermatozoa are essential at the outset and during tests.

SUMMARY

1. Sperm should be collected directly into clean glass vials.

2. It should not contact rubber condoms, stoppers, or bulbs even for a short interval. Contact with brass and copper should also be avoided.

3. Contact with air has no demonstrable effect. Temperature should be kept constant during an experiment as variations affect the spermatozoal longevity. Sperm should be preserved at temperatures approximating 5° C. for reduced aging effect.

4. Sperm collected from the vagina age more rapidly than the control. Urine, pus, and bacteria are deleterious.

5. Carefully planned controls should be run concurrently with all tests on spermatozoon vitality.

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A SIMPLE APPARATUS FOR DETERMINING PERIPHERAL ARTERIAL PRESSURE IN THE RABBIT'S EAR*

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THE apparatus described below is designed to determine the pressure of the central artery of the rabbit's ear. Devices similar in principle have been described, notably that of Grant and Rothschild (1934). However, in our hands results with this simpler apparatus have been uniform when the indicated precautions were observed.

A glass reservoir (Fig. 1) (*A*) containing mercury is connected by means of rubber tubing with a one-half inch T tube (*B*). The lower arm of the T (*C*) contains a lucite rod of proper length which serves to transmit light from the source (*D*) and also to provide a seal for the lower portion of the air chamber. The open end of the upper arm of the T is covered by a small sheet of condom rubber which is securely fastened around the neck of the tube. Care must be taken not to stretch the rubber while fastening in place. Over the top of the rubber-covered chamber is placed a plate of glass (*E*), about 1 inch by 4 inches by ¼ inch. This glass is attached to a screw (*F*) so that it may be moved freely up and down over the air chamber. When in use, the shaved ear of the rabbit (the animal anesthetized or held in a suitable holder) is permitted to rest by its own weight on the rubber-covered top of the air chamber. The glass plate is then screwed down over the ear, holding it firmly but gently enough not to compress the artery. By observing from above, the position of the ear is easily adjusted so that the artery lies across the diameter of the chamber tube. A binocular dissecting microscope (25 diameters) supported above the plate (*E*) facilitates adjustment and observation of the artery. By means of the needle valve (*G*) the zero point is adjusted on the mercury manometer (*H*). The mercury bulb is then raised by means of a suitable screw and ratchet, which balloons out the rubber diaphragm covering the inflation chamber and compresses the artery between it and the glass plate above. When the artery is seen to be just occluded the manometer is read. By carefully adjusting the occluding pressure it is possible to measure both diastolic and systolic pressures. Several readings on one animal may thus be taken in rapid succession, but it is essential to take successive readings on the same point on the artery. If these readings diverge greatly it may indicate leakage or other defects in the apparatus, provided both the environmental and animal temperatures remained constant and the animal was not restless.

The apparatus is constructed of ⅜ inch pressure tubing, and great care must be taken to see that all connections are airtight. The most frequent point of leakage is the rubber diaphragm. It is desirable to check this each time the instrument is used. The whole should be suitably supported and firmly fastened to the table top.

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A suitable rabbit holder may be made by shaping plaster of paris bandages about a model which is then cut into an upper and lower section. A rabbit placed in a crouching position in the lower half is held firmly but in a comfortable position by strapping down the upper section. The ears protrude through an opening in the top. The rabbit quickly learns to remain perfectly quiet for the short period necessary to obtain manometer readings.

The apparatus has also been used successfully with guinea pigs and rats.

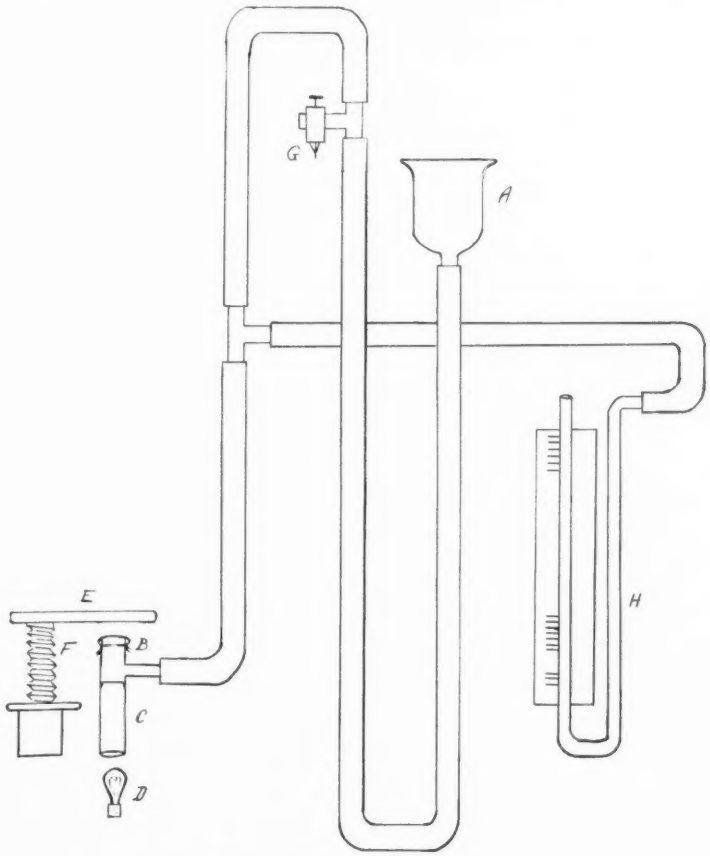

Fig. 1.—Explanation in text.

Although the readings obtained with this apparatus were found to be consistent and reproducible under controlled conditions, it was desirable to compare the readings on the peripheral pressure so obtained with the systemic arterial pressure as obtained through the femoral or carotid arteries. This procedure was also expected to serve as an accurate check on the efficiency of the apparatus. Several rabbits were tested in this way. On the anesthetized animal, a carotid cannula was inserted and connected with a *U* tube mercury manometer. Simultaneously readings were taken on the ear artery with the apparatus described above. The two pressures were recorded at the same moment by different observers. Readings were first taken on the anesthetized animal at about one minute intervals for fifteen to thirty minutes. Later the animal

TABLE I

SIMULTANEOUS READINGS ON CAROTID AND PERIPHERAL (EAR) ARTERIAL PRESSURES, IN MM. OF MERCURY. RABBIT. ANIMAL NO. 10

TIME	CAROTID	PERIPHERAL
2.05	68	22
2.06	70.5	23
2.07	63.5	19
2.08	67	20
2.10	67	19.5
2.11	70	21.5
2.12	67.5	21
2.14	69	21
2.15	69.5	21.5
2.16	68	20
2.17	71	21.5
2.19	68.5	19
2.21	71	21
2.22	69	20
2.23	68	19
2.24	67.5	17
2.25	68	18.5
2.26	67	17
2.27	67.5	18
2.28	69	20.5
2.29	69.5	21
2.31	69	20.5
2.32	70	21.5
2.33	69.5	21
2.34	68.5	19
2.35	69	20
2.37	69	20.5

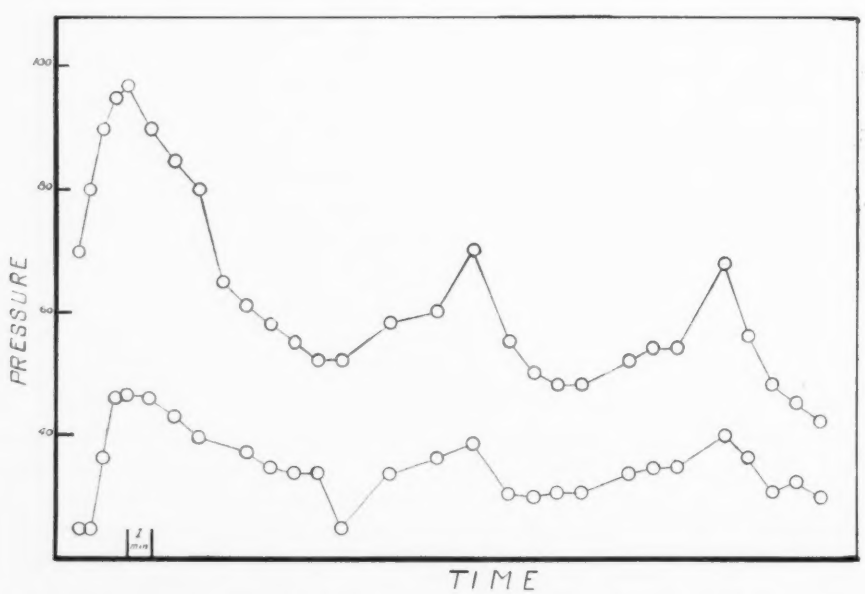

Fig. 2.—Pressure of carotid artery (upper curve), central artery of ear (lower curve), in mm. of mercury. Simultaneous readings. 1.1 c.c. pitressin intravenously one minute before first reading. Rabbit. Animal No. 14.

received pitressin intravenously and both carotid and peripheral pressures were again recorded. Table I gives the data on these readings over a thirty-five minute period for one animal. In Fig. 2, the data show graphically the carotid

pressure (upper curve) and the peripheral pressure (lower curve) after the animal received 1.1 c.c. pitressin two minutes previous to the first reading.

The striking similarity in form of the two curves would seem to indicate the efficiency of the apparatus in measuring peripheral arterial pressure, as well as to show that under these conditions the peripheral arterial pressure so obtained follows the same fluctuations shown by the carotid pressure although of lower amplitude.

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COMPARISON OF PROCEDURES FOR STAINING TUBERCLE BACILLI IN FLUORESCENCE MICROSCOPY*

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IN THE detection of tubercle bacilli, uniformly better results have been reported with fluorescence than with the Ziehl-Neelsen stain, the figures ranging from 6 per cent to 40 per cent more positive tests for the former method. The advantages given for fluorescence microscopy are that it stains more bacteria; it is a more rapid method of determination; it is less tiresome for the laboratory technician; and it offers better contrast of bacilli in the field of vision.

Following the early reports of Hagemann[2, 3] in 1937 and 1938, there developed widespread interest in this procedure. Because some investigators have altered the staining technique in some respects, a comparative study of these procedures was felt to be desirable. An experiment was therefore conducted to test the value of various decolorizers and counterstains used in fluorescence microscopy.

APPARATUS

Light Source.—A simple 100 watt mercury vapor lamp placed in a lamp housing and used in conjunction with a special blue glass filter was employed as the source of ultraviolet light radiation.

Microscope.—An ordinary monocular microscope was used with a 4 mm. objective N.A. 0.85 together with a 10x ocular. A preliminary comparison of 4 mm. objectives N.A. 0.85 and N.A. 0.66 revealed the former as having a brighter field of vision, although less contrast between the organisms and their background was apparent. The latter type of objective appears to reduce the transmission of light about 60 per cent according to Richards et al.[6] An aluminized mirror was placed over the mirror of the microscope to avoid loss of ultraviolet light rays, and a yellow filter was inserted into the eyepiece.

PROCEDURE

Smears.—Slides of equal thickness were prepared with smears made from a uniform saline suspension of tubercle bacilli. All slides were examined within twenty-four hours after staining, since fluorescence begins to be reduced thereafter.

Dye.—Auramine in a 0.1 per cent aqueous solution with the addition of 5 per cent phenol was used. All slides were steamed for five minutes and then washed in tap water.

Decolorizers.—Five different decolorizing solutions were used in several strengths and for the time periods as follows:

*From the Laboratory Dept., Los Angeles Sanatorium, Duarte, Calif.
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Decolorizer	*Time Used*
3 per cent concentrated hydrochloric acid in 95 per cent alcohol	Until preparation colorless (ab. 5 min.)
3 per cent concentrated hydrochloric acid in 70 per cent alcohol	Until preparation colorless (ab. 5 min.)
0.5 per cent concentrated H.C.L. and 0.5 Gm. NaCl in 70 per cent alcohol	3 min. (1 min. pour off and renew for 2 min.)
5 per cent concentrated nitric acid in 70 per cent alcohol	30 seconds
25 per cent concentrated sulphuric acid in 70 per cent alcohol	30 seconds

Further Staining.—

Potassium Permanganate Solution.—A series of slides was stained with and without the use of potassium permanganate solution in order to demonstrate its importance in eliminating side fluorescence. A 1:1000 aqueous solution was prepared, into which slides were dipped for one second following decolorization and washing in tap water.

Methylene Blue Solution.—Other slides were counterstained with methylene blue solution and compared with smears untreated with this dye, so that its value in offering better contrast between the bacilli and their background could be studied. Loeffler's alkaline methylene blue solution diluted 1:10 was used for three seconds following decolorization and in some cases treatment with potassium permanganate solution. The slides were then washed in running water and dried.

There were a total of twenty fluorescence-stained slides in this study. The presence of tubercle bacilli on each of the slides was verified by a control smear stained by the Ziehl-Neelsen method. Each fluorescence-stained slide was examined for best visibility of tubercle bacilli as compared with other slides in the series. The intensity of light radiation offered by the organisms as they appeared to the eye of the investigator was the deciding factor in establishing the degree of luminescence on microscopic examination.

RESULTS

A slide decolorized with a solution of 25 per cent concentrated sulphuric acid in 70 per cent alcohol followed by staining with a 1:1000 aqueous solution of potassium permanganate offered the best visibility. Another slide decolorized with a solution consisting of 3 per cent concentrated hydrochloric acid in 70 per cent alcohol and counterstained with potassium permanganate solution and the methylene blue solution presented a degree of visibility which proved second best. A slide decolorized with a solution of 0.5 c.c. concentrated hydrochloric acid and 0.5 Gm. sodium chloride in 70 per cent alcohol and counterstained with methylene blue solution gave the third best results.

In studying those slides which were not stained following decolorization, the investigator was troubled somewhat by side fluorescence of material such as crystals, tissue cells, fibers, secretions and like particles. However, it was generally observed that somewhat fewer organisms were present on those slides which were counterstained with methylene blue solution.

DISCUSSION

There is no doubt that an improved source of light which would allow for binocular vision would greatly improve detection of the organisms. Until this is found we must necessarily restrict our improvements to bettering the staining technique.

The dye, auramine O, appears to be the best in use at present. A comparison of this coloring matter with other fluorescent dyes such as fluorescein, rhodamine, tartrazine, chrysoidine and Victoria green showed that none of these could equal auramine in degree of fluorescence when exposed to ultraviolet rays. Hagemann[3] cold-stained his slides at room temperature as did Richards et al., but we preferred the steam-staining period of five minutes as used by Hermann[4] and by Bogen.[1] We felt better assured of thorough fastness of the stain and a lesser effect on them by strong decolorizing solutions which were subsequently used.

In decolorization of his stained slides Hermann used a solution of 3 per cent concentrated hydrochloric acid in 70 per cent alcohol for a period of fifteen to twenty seconds. He then made the observation that if this solution was used for a period longer than three minutes, some of the tubercle bacilli also became decolorized. Thompson,[7] using a decolorizing solution of 1 per cent concentrated hydrochloric acid and 0.5 Gm. sodium chloride in 70 per cent alcohol, treated his stained slides for a period of twenty minutes (ten minutes, pour off, and renew ten minutes). He preferred this stronger acid solution and a longer period of decolorization than that used by Richards from whom Thompson's technique was taken. From the results of these observations and those of this experiment, the stronger decolorizing agents appear to improve the fluorescence of the organisms and prevent interfering side fluorescence. Nevertheless, as stated above, care must be used in accurately timing the decolorizing period to avoid bleaching of the tubercle bacilli.

Fewer organisms are seen with the use of a dye counterstain such as methylene blue. This has been noted also by other investigators and is important on slides with scanty bacilli. Therefore, the use of potassium permanganate solution following decolorization can be sufficient in overcoming interfering fluorescence.

According to Oscarsson,[5] "overdiagnosis" of the slide must be cautioned against, especially in cases with few bacilli present on the slide. This could result from fluorescence of near-by material resembling the morphology of acid-fast bacilli. We found this to be true in our early studies with 100 comparative slides. There was a tendency to interpret a very high percentage of positive results although these subsequently proved to be unfounded in great part when checked by standard control procedures. We are still unable to feel complete reliance in this procedure as an accepted method of examination. Assurance in the diagnosis of tubercle bacilli by fluorescence microscopy is not easily acquired.

SUMMARY

Lacking improvement in the source of ultraviolet light and in the luminescing dye used in fluorescence microscopy, changes can be suggested in the

use of various types of decolorizing and counterstaining solutions for the detection of tubercle bacilli.

The application of stronger decolorizing agents than those commonly employed appears to be advantageous.

The additional staining of the slides with potassium permanganate solution improves the visibility of the organisms.

Further counterstaining with methylene blue solution seems to be an unnecessary step and may only reduce the number of organisms visible in the field.

Confidence in the use of this procedure is not easily gained.

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CHEMICAL

THE MICRODETERMINATION OF AMINO ACID NITROGEN IN BLOOD WITH THE SPECTROPHOTOMETER AND WITH THE OPTICAL COLORIMETER*

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RECENTLY a modification of Folin's[1] method for the determination of amino acids in the blood using the optical colorimeter was published by Sahyun.[2] He used Danielson's[3] mixed amino acid standard consisting of glycine and glutamic acid. The major change from the Folin method was that the amino acid solution (or the blood filtrate) was heated with the dye in a boiling water bath for three minutes instead of being left in the dark overnight. This materially accelerated the determination of amino acids. Both a macromethod and a micromethod were proposed, but it was found that the color produced in the micromethod was too light for accurate optical colorimetric or spectrophotometric determination. The present study was undertaken in an effort to increase the intensity of the color difference, and hence the accuracy of the method, and to adapt it to the spectrophotometer.

EXPERIMENTAL

Reagents.—

Sodium Hydroxide, 0.05 N solution.

Sodium Borate, 2 per cent solution.

Phenolphthalein, 0.25 per cent solution.

Sodium-β-naphthoquinone-4-sulfonate, 0.5 per cent solution prepared freshly before using. Do not use a solution that has stood for more than 15 or 20 minutes.

Special acetic acid-acetate reagent, 100 c.c. of 50 per cent acetic acid diluted with an equal volume of 5 per cent sodium acetate.

Sodium thiosulfate, 4 per cent solution of $Na_2S_2O_3.5H_2O$.

Stock standard amino acid solution, containing 0.1 mg. of alpha amino nitrogen per c.c. Samples of glycine and proline are dried to constant weight over sulfuric acid in vacuum. Accurately weigh 187.8 mg. of proline and 412.8 mg. of glycine, and dissolve in 0.07 N HCl containing 0.2 per cent sodium benzoate as a preservative. The

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resulting solution is made up to 1 liter in a volumetric flask, using 0.07 N HCl—0.2 per cent sodium benzoate as a diluting fluid.

Working standard amino acid solution, containing 0.004 mg. alpha amino nitrogen per c.c. Prepared by accurately introducing 2 c.c. of the stock standard into a 50 c.c. volumetric flask and diluting to volume.

Dilute tungstic acid solution, 40 c.c. of 10 per cent sodium tungstate in a liter volumetric flask diluted to about 800 c.c. with water. Add 40 c.c. of ⅔ N sulfuric acid and dilute to volume. This solution will not keep indefinitely; and whenever a white precipitate of tungstic acid appears in the bottom of the bottle, a fresh solution should be made up.

Procedure.—A Model 11 Coleman Spectrophotometer was used. The optimum wave length for this method on the spectrophotometer was found to be 470 mμ.

Using a calibrated micropipette, introduce 0.2 c.c. of fingertip or oxalated blood into 6 c.c. of the special dilute tungstic acid solution in a 15 c.c. centrifuge tube. Mix well, let stand for about five minutes, and centrifuge. Into a graduated test tube, introduce 5 c.c. of the filtrate and simultaneously measure 5 c.c. of distilled water into another graduated test tube for the blank. Add to each 1 drop of phenolphthalein and neutralize to a definite pink with 0.05 N sodium hydroxide. If on standing a short time the color gradually fades, do not add more, as the color will return when the borate is added. Add 1 c.c. of 2 per cent sodium borate; the color should be of a uniform pink shade in all of the tubes. Then add 0.4 c.c. of freshly prepared sodium-β-naphthoquinone-4-sulfonate and mix well by shaking. Immerse in a boiling water bath for three minutes, cool under running water. Add 1 c.c. of acetic acid-acetate reagent followed by 1 c.c. of 4 per cent sodium thiosulfate. Dilute to the 10 c.c. mark, mix by shaking (do not invert unless the tube has a ground glass stopper), and compare colors.

Standardization of the Spectrophotometer.—The 0.004 mg. amino acid standard was used to standardize the spectrophotometer by taking the following portions:

A blank consisting of 5 c.c. of distilled water
1 c.c. of standard, corresponding to 2.48 mg. per 100 c.c.
2 c.c. of standard, corresponding to 4.96 mg. per 100 c.c.
3 c.c. of standard, corresponding to 7.44 mg. per 100 c.c.
4 c.c. of standard, corresponding to 10.02 mg. per 100 c.c.
5 c.c. of standard, corresponding to 12.40 mg. per 100 c.c.

All samples were diluted to 5 c.c. with distilled water and treated the same as the blood filtrates. The per cent absorption was graphed against the mg. per 100 c.c. concentration in the blood on semilogarithmic paper. Then for all determinations, a blank was used instead of a standard, and the graph was consulted for the concentration.

Optical Colorimeter.—When this method was tried on the optical colorimeter, it was found to give results which were 0.2 to 0.5 mg. per 100 c.c. less than on the spectrophotometer. The 3, 4, and 5 c.c. standards, as described under standardization, were used with the standard set at 20 mm.

DISCUSSION

In adapting the macromethod to the spectrophotometer, it was found that the only modification necessary was to reduce the amount of 0.25 per cent solution of sodium-β-naphthoquinone-4-sulfonate from 2 c.c. to 1 c.c. for each sample. The standardization of the instrument for the macromethod was carried out using portions corresponding to 3.33, 6.67, 10.0, 11.7, and 13.3 mg. per 100 c.c. in blood of either the macrostandard of glycine-glutamic acid as in Sahyun's original macromethod[2] or a macrostandard made from a stock proline-glycine standard.

In the micromethod the principal changes made consisted of a reduction in the amount of dye used, an increase in the concentration with a decrease in its volume of the precipitating reagent, and the substitution of a proline-glycine standard for the glycine-glutamic acid standard.

Recovery experiments were made by adding known amounts of glycine-glutamic acid standard in three different concentrations to blood filtrates of both the micromethod and the macromethod. The average deviation from the theoretical recovery of added glycine-glutamic acid standard in nine experiments was −0.33 mg. per 100 c.c. for the micromethod and −0.14 mg. per 100 c.c. for the macromethod.

A series of 24 oxalated bloods run to test the accuracy of the micromethod as compared to the macromethod showed an average difference of +0.054 mg. per 100 c.c. In most cases the variation amounted to 0 to 0.3 mg. per 100 c.c., and in no case was it greater than 0.7 mg. per 100 c.c. The amino acid nitrogen levels (fasting) ranged from 6.6 mg. per 100 c.c. to 11.4 mg. per 100 c.c., with an average of about 8.

Glutamic acid, glycine, alanine, valine, leucine, phenylalanine, tyrosine, proline, histidine, cystine, arginine, and tryptophan were made up so that 1 c.c. of solution would contain 0.1 mg. of alpha amino nitrogen. From these solutions, mixtures were made up which simulated as nearly as possible the amino acid compositions of casein, lactalbumin, and globin as reported in the literature,[4] because it was felt that these would simulate the amino acid composition of the blood. Proline, in spite of the fact that it has no primary alpha amino group, reacts with the dye and so was added in correct concentration to the mixtures. Micro-Kjeldahl analyses were made of all of the amino acid solutions and were found to agree closely with the theoretical.

Because it was found that proline did react with the dye, pseudoprotein mixtures were studied with and without proline present. It was found that proline had an effect greater than its nitrogen concentration as compared to glycine and glutamic acid. This effect seemed to be enhanced by the presence of arginine and presumably by other basic amino acids.

In Fig. 1 the micromethod calibration curves on the spectrophotometer at wave length 470 m/^{u} are shown for the two representative pseudoprotein

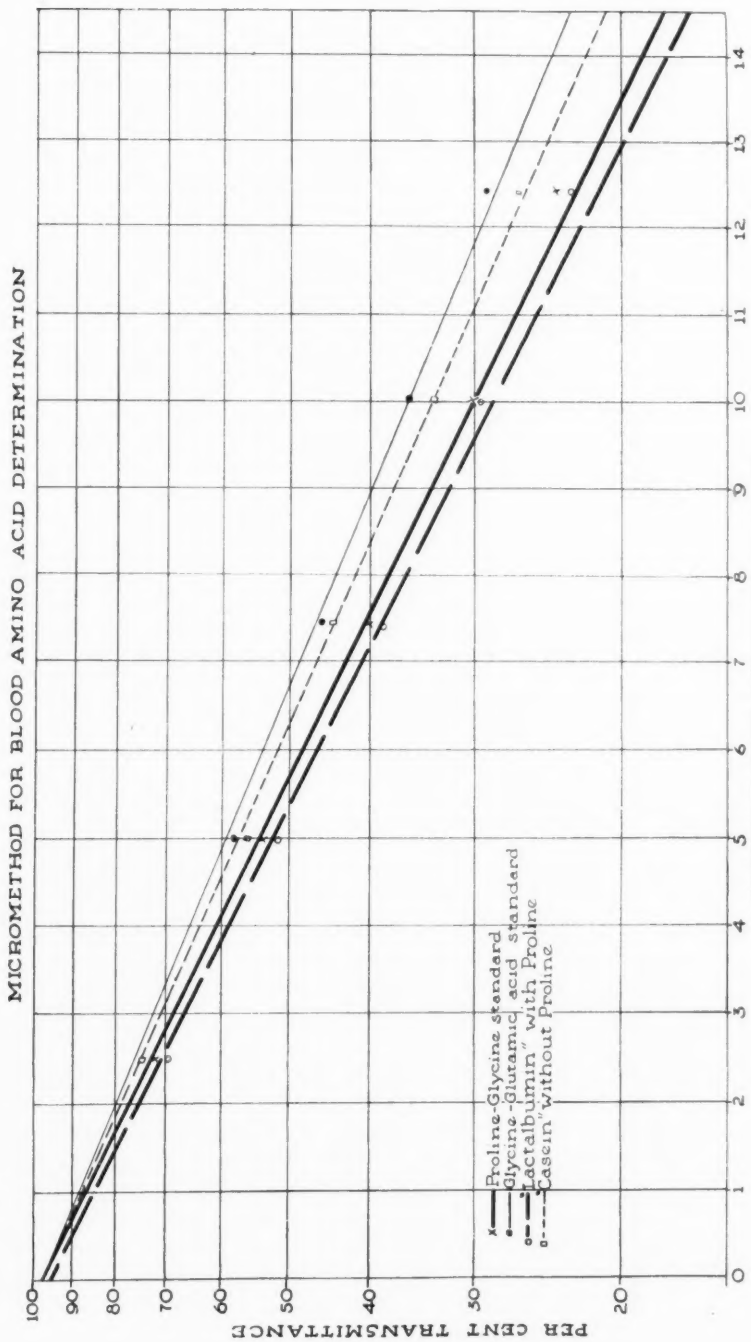

Fig. 1.—Calibration curves for amino acid micromethod using different amino acid mixtures.

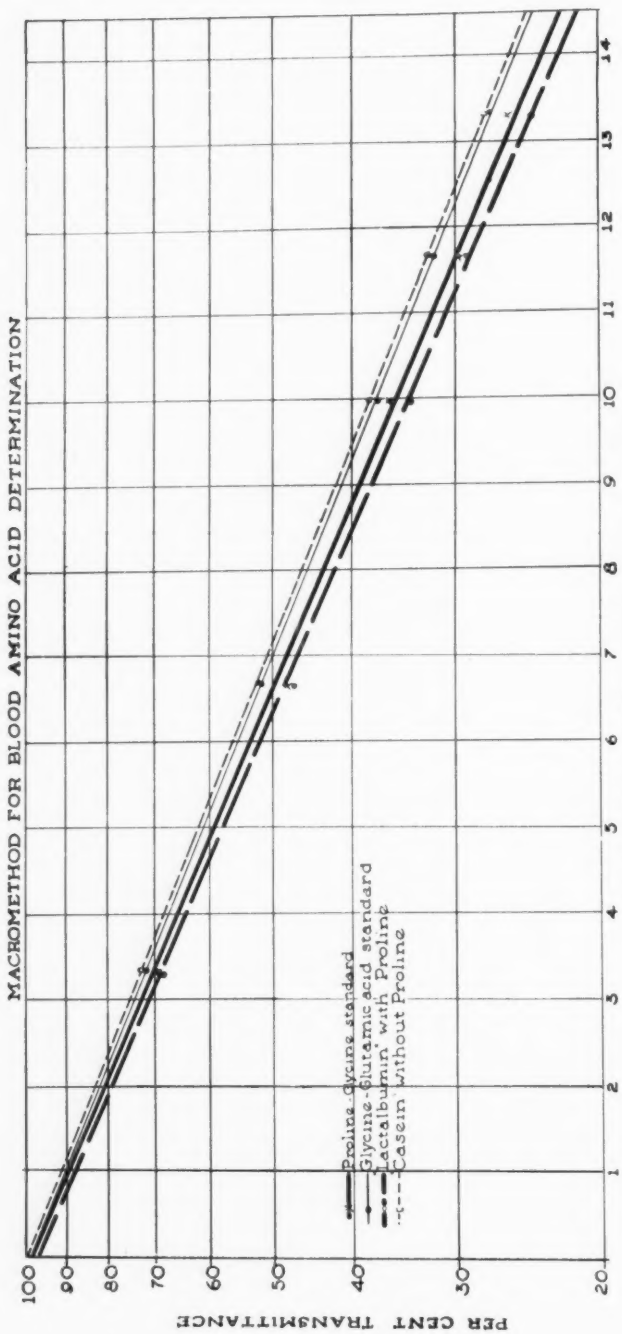

Fig. 2.—Calibration curves for amino acid macromethod using different amino acid mixtures.

mixtures giving respectively the least and the greatest absorption. For comparison the curves for the usual glycine-glutamic acid standard and for the new proline-glycine standard are also pictured. In the proline-glycine standard, 2 parts of the alpha amino nitrogen are due to proline and 7 parts to glycine. Fig. 2 shows similar comparative curves for the macromethod. It can be seen that the proposed proline-glycine standard is more nearly a median between the pseudoprotein extremes in both the micromethod and the macromethod than is the glycine-glutamic acid standard.

SUMMARY

A new micromethod for the determination of amino acid nitrogen in the blood, based on the Sahyun modification of Folin's method is described. Various factors which increase the accuracy over the previous micromethod are taken into consideration. The method is adaptable both to the spectrophotometer and to the optical colorimeter.

The author wishes to acknowledge her gratitude to Dr. Melville Sahyun of Frederick Stearns and Company for his helpful suggestions and to Dr. Hans Hecht of the William J. Seymour Hospital for technical assistance in obtaining the blood samples.

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A NOTE ON A SIMPLIFIED AND RAPID TEST FOR PROTEINURIA

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With the Technical Assistance of Charles D. Boyd, U. S. Army

IN THIS laboratory large numbers of routine urinalyses are performed daily. A test for proteinuria which would involve a minimum of equipment, a simple technique, and accurate results was sought.

The procedure developed represents a qualitative ring test for detection of proteinuria which, by observance of the degree of density of the ring, is roughly quantitative.

The reagent, developed by one of us (C. D. B.), consists of a mixture of equal parts of a 5 per cent aqueous solution of trichloracetic acid and absolute methyl alcohol. This reagent, in well-stoppered dark bottles, may be kept indefinitely without evident deterioration. The trichloracetic acid precipitates the urinary protein, and methyl alcohol makes the resulting solution light enough to overlay the urine.

The test is performed as follows: From 5 to 10 c.c. of urine are centrifuged in a centrifuge tube, or ordinary test tube, and this is overlaid with the reagent by dropping four to eight drops from a dropper bottle. The presence of an abnormal amount of albumin is indicated by the prompt formation of a white ring at the junction of the urine and reagent. The most consistent results are obtained when the test is read between thirty to sixty seconds. Following the reading of the test, the urine (and reagent) may be poured out and the microscopic examination of the urine carried out on the sediment.

The advantages of this technique over those in general usage are most evident in a laboratory where a large volume of work is done such as in military hospital laboratories. The procedure is time saving in that no complicated techniques are involved; one centrifugation and one set of test tubes serve for both the protein test and microscopic study. The simplicity of performance and the distinctness of positive tests make it a dependable test for both student and trained technicians.

Trichloracetic acid is recognized as an efficient reagent for precipitating protein and has been applied to the study of urine in the form of both contact and turbidity tests. Various studies, however, were carried out by the authors to obtain the optimum strength for this particular technique. Studies on both pathologic urine specimens and specimens prepared by the addition of varying quantities of blood serum indicate that the small quantities of protein normally present in the urine are not detected by this test, and amounts generally conceded to be pathologic show positive tests.

This test has been used in an Army Station Hospital laboratory as the routine method for testing for proteinuria for five months, during which time over 4,000 tests have been made. Most of the positives were checked by other

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techniques, and on a considerable percentage, quantitative determinations were done. Only one false positive was obtained, this being due to an excessive amount of mucus which was not thrown down by centrifugation. Two hundred consecutive urines negative for protein by the authors' technique were also negative with Roberts' reagent.

Studies of the microscopic sediment from tubes in which this test has been performed show no alteration in either the formed elements or unorganized chemical constituents. As a matter of fact, studies show that the pH of the urinary sediment is not altered.

SUMMARY

A simple, time- and equipment-saving contact test for proteinuria using trichloracetic acid and methyl alcohol has been described. We believe this test will prove to be of considerable value, especially in military laboratories.

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BOOK NOTICES

The Neuromuscular Maturation of the Human Infant*

THE author has demonstrated, within a little over a hundred pages, what to expect in the development of the newborn infant until it arrives at a state where its activities are under cortical control.

The maturation of the infant shows its development to cover periods of transition from a phase where the body activities are under subcortical control to one in which the activities are under cortical control. Through diagrams and illustrations as well as descriptive text, the various phases the infant passes through from subcortical to cortical control are aptly demonstrated.

In describing the infant's growth the author gives the various phases the infant passes through in learning to roll over, crawl, sit, and walk. Diagrams are presented to chart these phases of development so that one infant's activity can be compared with that of another and normality for an individual can be determined.

The author in concluding states that exercise of a newly developing function is inherent in the process of growth, and if ample opportunity is afforded at the proper time, specific achievements can be advanced beyond the stage normally expected. She also concludes that training of any particular activity before the neural mechanisms have reached a certain state of readiness is futile.

After reading the book, which was found to be very instructive and entertaining, the reader feels that he has a much better insight into the neuromuscular maturation of the infant.

L. E. S.

The Autopsy†

THE autopsy is the subject of a special war number of the *Journal of Technical Methods* and the *Bulletin of the International Association of Medical Museums.*

The importance of the autopsy along with special bacteriologic, chemical, and other methods in furthering our knowledge of war wounds, diseases of war, and tropical medicine is emphasized in an introduction by the Conference Group on Pathology of the National Research Council.

There follows a very complete discussion of the autopsy, including autopsy permission, the relation of the coroner, regulations of the Army and Navy, equipment and supplies necessary for the autopsy room, and the technique of the autopsy. The techniques described along with the description of the protocol are those usually to be found in the better institutional departments of pathology. Toxicologic, bacterial, and medicolegal examinations are also discussed.

The presentation should serve as an excellent means of securing standard performance of autopsies in the Army and Navy and should prove of value to many pathologists in maintaining the standard of the autopsy.

J. H. S.

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*The Neuromuscular Maturation of the Human Infant. By Myrtle B. McGraw. Cloth, 140 pages. Morningside Heights, Columbia University Press, New York, 1943.

†The Autopsy. Special war number of the Journal of Technical Methods and Bulletin of the International Association of Medical Museums, No. XXIII, February, 1943. Cloth, 85 pages. Waverly Press, Inc., Baltimore, Md., 1943.